EUROPEAN CONGRESS OF TOXICOLOGIC PATHOLOGY

20TH EUROPEAN CONGRESS OF TOXICOLOGIC PATHOLOGY

26-29 SEPTEMBER 2023 | BASEL, SWITZERLAND

EMERGING THERAPEUTIC MODALITIES

ABSTRACT BOOK

DEAR COLLEAGUES AND FRIENDS

Grüezi mitenand! Welcome to the city of Basel and to the 20th European Congress of Toxicologic Pathology! The Scientific and Local Congress Organizing Committees are delighted to host this meeting in Basel - well-known for its life science industry and research cluster. Basel is also a city full of history, science and art. The oldest traces of settlement date back to the middle Paleolithic period (about 130,000 years ago) and were found on the banks of the Rhine, where the Novartis campus is now located. Basel and the surrounding area were also important Roman settlements.

The University of Basel was founded in 1460 and it was the first on Swiss territory. Renowned intellectuals like Erasmus from Rotterdam lived and worked in Basel and had their work published by innovative printers facilitated by state-of-the-art paper production at the time and testimony of an early and important knowledge cluster.

In the mid-16th century, many immigrants arrived from Italy and France often due to religious reasons. These newcomers brought with them a diverse array of skills such as knowledge in silk trade, dyeing, and weaving. In the 19th century, the dye industry (aniline) by Alexander Clavel and the J. R. Geigy pharmacy marked the beginning of the chemical-pharmaceutical industry in Basel. Alexander Clavel later sold his company to evolve first to Ciba (chemical industry Basel) and then to Ciba-Geigy.

Given the special place that the pharmaceutical industry enjoys in Basel, the topic of this year's ESTP congress "Emerging Therapeutic Modalities" tries to do it justice by covering a range of state-of-the-art therapeutic modalities. On Tuesday morning, 26th of September and ahead of the start of the congress, the International Academy of Toxicologic Pathology (IATP) is hosting a half-day workshop on safety assessment of medical devices including regulatory requirements. On Tuesday afternoon, the ESTP congress will officially begin at 13.30 with a keynote lecture on the current medicine toolbox and the question of what the "Next Big Thing" will be. The congress will conclude on Friday 29th of September, with a series of case presentations. In between, there will be sessions on nucleic acid technologies, gene and cell therapies, innovative biologics such as bispecifics or antibody-drug conjugates, targeted protein degraders, immune-mediated toxicities and immunogenicity, and a panel of experts will discuss the future of carcinogenicity studies.
In addition to the scientific program, the ESTP congress is also an opportunity to award distinguished work by our colleagues like the ESTP Publication/Thesis award or the BSTP Chirukandath Gopinath Lecture Award.

We acknowledge the invaluable dedication and voluntary efforts of numerous ESTP members and the vital contributions of our professional congress organizer (PCO Partners in Congress Organisation). Without their professionalism, the organization of a congress of this size would not be possible. Furthermore, we are very grateful to all our sponsors and exhibitors for supporting once again the ESTP congress so generously. The latter are also important meeting points during the congress and their state-of-the-art applications and scientific solutions will be presented. Please make sure that you take full advantage of their presence and honor them by a visit at their booths and Sponsored sessions.

Finally and beyond pure science, we welcome you to meet and exchange ideas with your colleagues during the coffee breaks and poster exhibitions, welcome reception at Bar Rouge, and during the Congress Dinner at KLARA. If you still find some time, venture into the beautiful city of Basel - full of history and art!

Sincerely,

Enrico Vezzali, **Josep Monné** and **Kuno Würsch** (co-chairs)
On behalf of the Local and Scientific Organizing Committees

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GENERAL CONGRESS INFORMATION

PROGRAM

ABSTRACTS AND CASE PRESENTATIONS

SCIENTIFIC ORGANIZING COMMITTEE, LOCAL REPRESENTATIVES AND CONGRESS ORGANIZERS

Scientific Organizing Committee

Basel Assaf
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Raffaella Capobianco
Pierluigi Fant
Thierry Flandre
Renaud Fleurance
Anna-Lena Frisk
Babunilayam Gangadharan
Paul Germann
Armelle Grevot
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Kuno Würsch
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Representatives for the IATP

Johannes Harleman
Matthias Rinke

Local Organizing Committee

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Armelle Grevot
Josep Monne
Fernando Romero Palomo
Marco Tecilla
Enrico Vezzali
Kuno Würsch

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pco PARTNERS IN CONGRESS ORGANISATION

USEFUL INFORMATION

Venue | The ESTP Congress will be organized in the Hyperion Hotel Basel, Messeplatz 12, Basel, 4058, Switzerland
The Messe Basel is one of the most important trade fair centres in Europe. The site has an exhibition space of approximately 141,000 square metres. The Hyperion Hotel Basel is located directly adjacent to the trade fair centre, and is thus ideal for trade fair visitors and conference guests. On the third floor of the 105-metre Messe Basel tower, 8 seminar and meeting rooms with ceiling heights of up to 5.5 metres are available for events. The light, bright meeting and events centre covers approximately 1,000 square metres, and is suitable for events of all kinds.

Registration Desk | The registration area in the conference centre will open for registration and questions on:
Tuesday 26 September: 07.30-18.00
Wednesday 27 September: 07.30-18.00
Thursday 28 September: 07.30-18.00
Friday 29 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 and to the exhibition area
- Daily lunch
- Daily coffee breaks
- Final programme
- Welcome reception
- Congress Dinner

WIFI | You will have free WIFI access on-site in the congress centre. Go to Settings -> WiFi and choose H_HOTELS_FREE_WLAN and follow the insctructions!

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.

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.

Congress rooms | The plenary lecture room is in room Geneva 1,2 and 3. The exhibition hall, posters and catering are located in the surrounding foyer, Geneva 4 and Amsterdam.

Poster Presentations | Posters will be exhibited during the entire congress in the foyer, room Geneva 4 and room Amsterdam. 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.

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

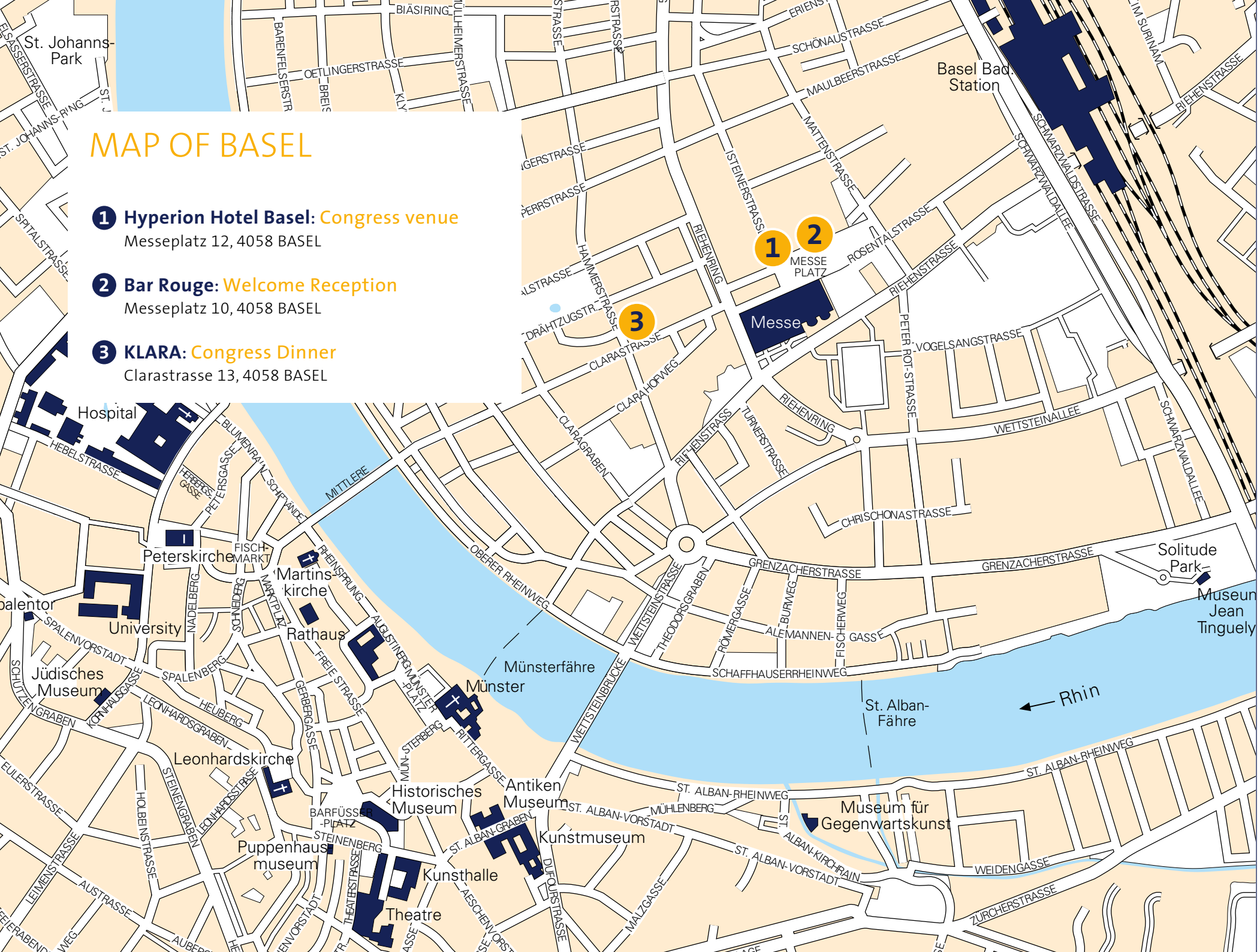
Anything lost? | Please go to the registration desk.

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.

MAP OF BASEL



1 Hyperion Hotel Basel: Congress venue
Messeplatz 12, 4058 BASEL

2 Bar Rouge: Welcome Reception
Messeplatz 10, 4058 BASEL

3 KLARA: Congress Dinner
Clarastrasse 13, 4058 BASEL

EXHIBITION FLOORPLAN

01 **AnaPath**
02 **BioTechne**
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04 **Instem**
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07 **Excilone**
11 **Resero Analytics**
12 **Proscia**
13 **Hammamatsu**
14 **Aira Matrix**
15 **Deciphex**
16 **Deepatholgy**

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.

Maronpot Guest Lecture

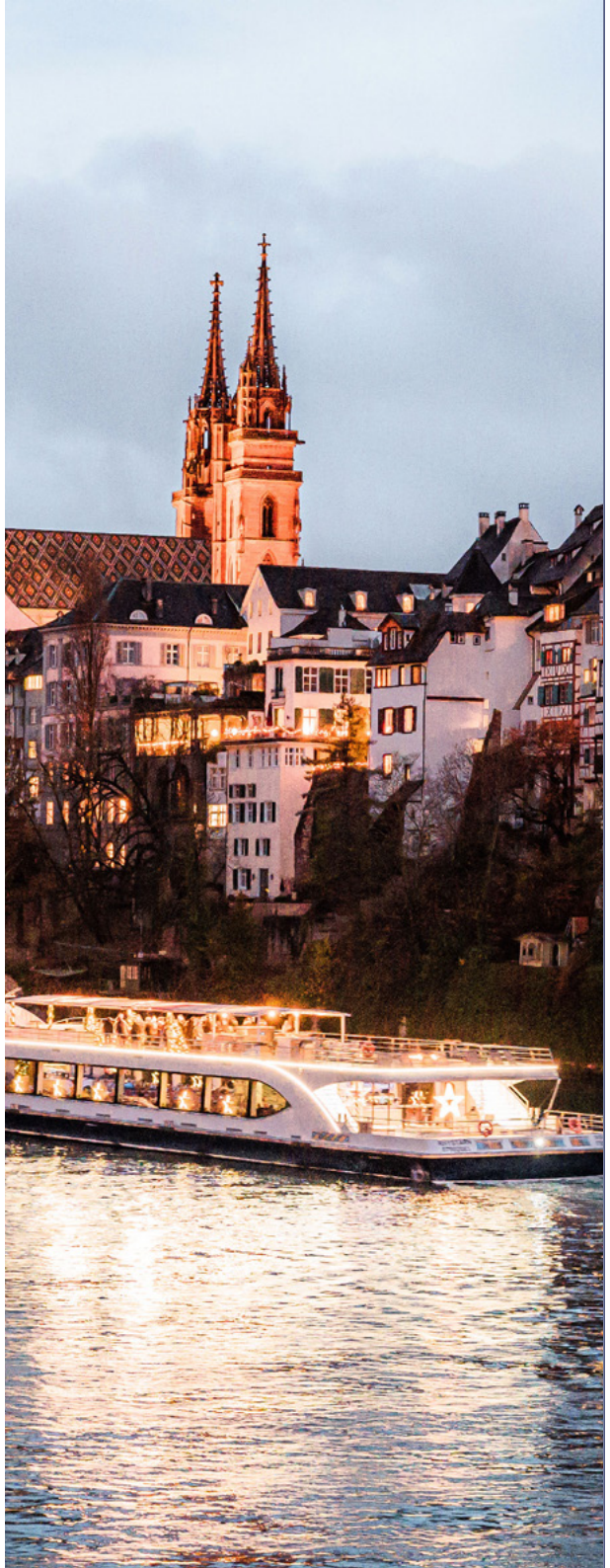
This award recognizes Dr. Robert Maronpot for his significant contributions to the field of toxicologic pathology and the advancement of the IATP. This lecture award is sponsored through an educational grant provided by The Telikicherla Higher Education Foundation.

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.



TUESDAY 26 SEPTEMBER 2023 | IATP SYMPOSIUM

Medical Device Safety Assessment: Pathology and Toxicology Perspectives

The global medical device (MD) industry continues to grow due to significant rise in use of medical devices as an interventional therapy or delivery tool. The preclinical development process of safety assessment of medical devices is unique, ever evolving to meet the regulatory requirements that are often different from that of other modalities. This symposium provides an overview of the current status, recent advances, and future trends on the following topics in Medical Device Safety Assessment.

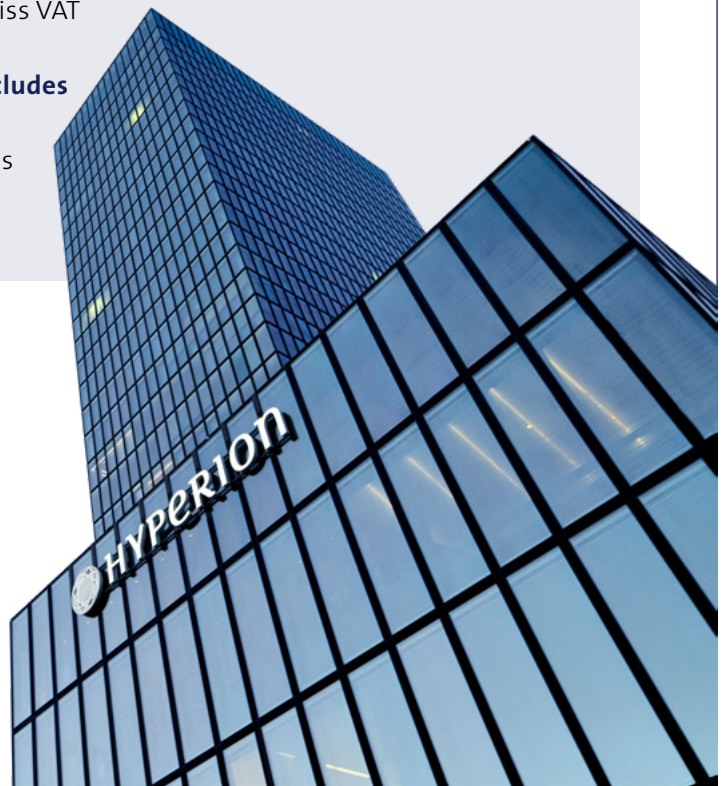
- Toxicology perspectives on medical device safety assessment.
- Fundamentals and challenges associated with microscopic evaluation and biocompatibility.
- Artificial Intelligence (AI) and Machine Learning (ML) for image analysis and interpretation of MD-associated pathological findings.
- Novel applications of currently existing MD for emerging therapeutic modalities.
- Extractables and leachables.
- Regulatory requirements and differences with various agencies.

The Hyperion Hotel
The IATP Symposium will be organized in the Hyperion Hotel in Basel, Messeplatz 12, 4058 Basel Switzerland

Registration fee
IATP Symposium € 180
Prices are incl. 7,7% Swiss VAT

The registration fee includes

- Admission to the IATP scientific sessions
- Coffee break
- Lunch



TUESDAY 26 SEPTEMBER 2023 | IATP SYMPOSIUM

08.00-08.30 **Registration IATP Symposium**

08.30-08.40 **Welcome and Introduction IATP Education Committee** *Deepa Rao*

08.40-09.30 **Medical Device Toxicology: Modern Day Safety Evaluation for the Potential Biological Risks Associated with Medical Devices** *Nicole Soucy*

09.30-10.20 **Challenges in Biocompatibility and Regenerative Medicine: Focus on Bone Implant Pathology - a Workup, Shortfalls and Forecasts** *Antoine Alves*

10.20-10.50 **Extractable and Leachable Study for Medical Devices & their Toxicological Assessment** *Flora Wegener*

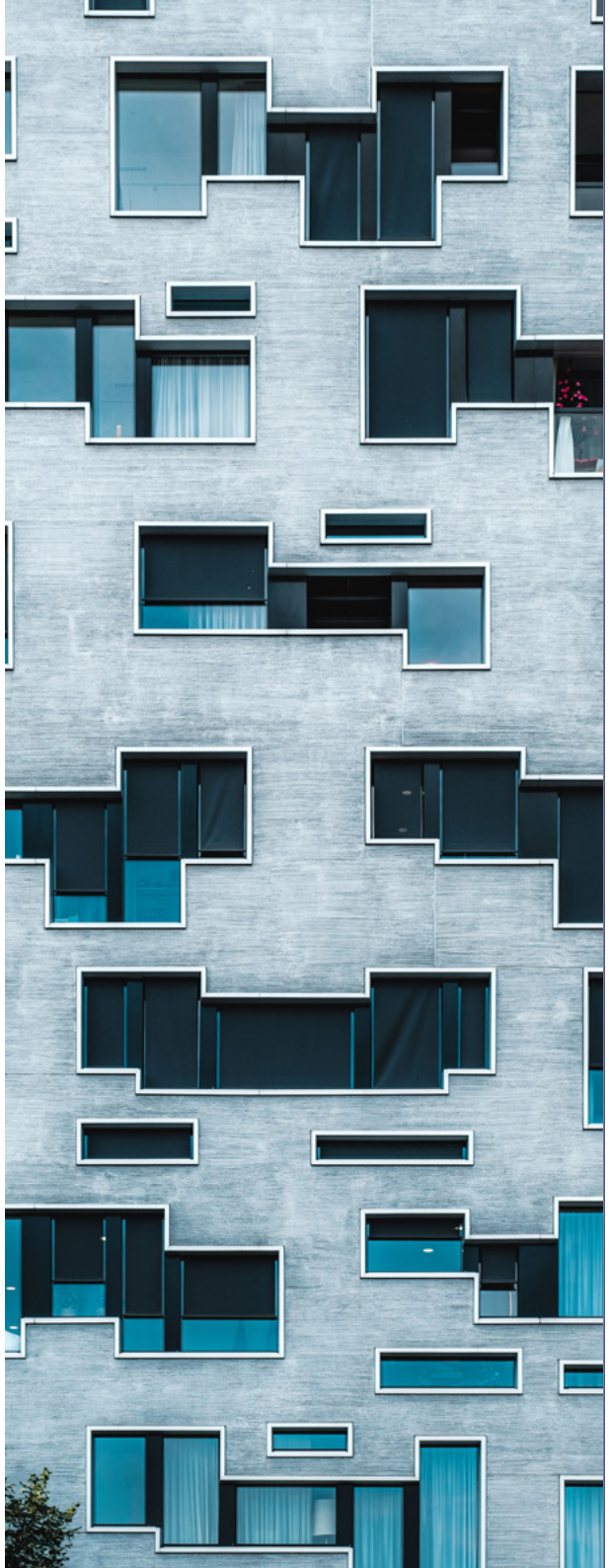
10.50-11.20 **Coffee break**

11.20-11.50 **Regulatory Requirements for Medical Devices regarding Toxicological properties** *Uta Bussmeyer*

11.50-12.25 **Panel Discussion**

12.25-12.30 **Closing Remarks IATP Education Committee**

12.30-13.30 **Networking lunch**



TUESDAY 26 SEPTEMBER 2023 | ESTP CONGRESS

12.30-13.30 **Networking lunch & Registration ESTP congress**

13.30-13.45 **Welcome ESTP Chair**

Congress opening session *Chairs: Fernando Romero & Josep Monné*

13.45-14.45 **Keynote Lecture: Building the medicine toolbox: how did we get here and what is the Next Big Thing?** *Richard Haworth*

Session: Nucleic acid technologies *Chairs: Fernando Romero & Josep Monné*

14.45-15.30 **The current state of nucleic acid therapeutics** *Roy van der Meel*

15.30-16.15 **Coffee break - Posters and Exhibition**

16.00-16.15 **Aiforia Sponsored session: Analyze, share and store studies effectively in AI-powered cloud environment** *Hanna-Kaisa Sihvo*

aiforia® AI for image analysis

Session: Nucleic acid technologies *Chairs: Pierlugi Fant & Raffaella Capobianco*

16.15-16.45 **Pathology findings associated with systemic and IT delivered ASOs in preclinical safety studies** *Kuldeep Singh*

16.45-17.15 **ASO-related morphologic findings and changes associated with intrathecal administration (IT) of drugs in nonhuman primates (NHPs) and rodents** *Annette Romeike*

17.15-18.00 **Regulatory aspects of preclinical oligonucleotide development** *Helen-Marie Dunmore*

18.15-19.30 **Welcome Reception - Bar Rouge**

AnaPath

WEDNESDAY 27 SEPTEMBER 2023 | ESTP CONGRESS

07.30-08.30 **Registration**

08.15-08.30 **Resero Analytics Sponsored session:**
Automated reporting for pathologists: data analysis, visualizations, and reporting with TurboToxicology *David Watson*

Session: Nucleic acid technologies *Chairs: Alok Sharma & Grazyna Wieczorek*
08.30-09.15 **mRNA platform approach for the preclinical development of vaccines** *Shambhunath Choudhary*

Session: New generation biologics *Chairs: Alok Sharma & Grazyna Wieczorek*
09.15-10.00 **Next generation bispecific antibodies and antibody fusion proteins for cancer immunotherapy** *Christian Klein*

10.00-10.30 **Coffee break - Posters and Exhibition**

Session: New generation biologics *Chairs: Anna Maria Giusti & Giovanni Pellegrini*
10.30-11.15 **Biologic Associated Immune-mediated Renal Disorders in nonclinical toxicity studies and their relationship to the clinical syndrome. of BAIRD** *Ken Frazier*

Maronpot Guest Lecture
Sponsored by IATP and the Telikicherla Higher Education Foundation (THE)

11.15-11.35 **Case Presentation: Morphology and Potential Mechanisms of Spinal Cord Findings Associated with ASO Administration in NHPs** *Jairo Nunes*

11.35-13.15 **Lunch - Posters and Exhibition**

12.45-13.15 **Deciphex Sponsored session: A Vision for GLP regulated Digital Pathology** *Colin Doolan*

Session: Gene and cell therapies *Chairs: Xavier Palazzi & Jan Klapwijk*
13.15-14.00 **The history and evolution of gene therapy** *Jonathan Appleby*
14.00-14.45 **The comprehensive pathologist toolbox for the preclinical development of AAV-based gene therapy products** *Basel Assaf*
14.45-15.30 **Liver gene transfer and editing: challenges and perspectives** *Alessio Cantore*

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

Session: Gene and cell therapies *Chairs: Basel Assaf & Armelle Grevot*
16.00-16.30 **General biopharmaceutical/regulatory/clinical aspects of Gene Therapies including rAAV vectors** *Andreas Hartmann*
16.30-17.15 **Development of PLX-R18 Cell Therapy as a Countermeasure for Hematopoietic Acute Radiation Syndrome** *Marianna Truman*
17.15-18.00 **Gene-modified differentiated lymphocytes (cell therapy)** *Jan Klapwijk*

THURSDAY 28 SEPTEMBER 2023 | ESTP CONGRESS

07.30-08.30 **Registration**

08.15-08.30 **Bio Techne Sponsored session: Characterizing Complex Tissues with Spatial Expression Analysis Using RNAscope, BaseScope and miRNAscope Technologies** *Vladimir Zlateski*

Session: New generation biologics *Chairs: Thierry Flandre & Josep Monné*

08.30-09.15 **ADCs - still emerging or coming of age?** *Kuno Würsch*

09.15-10.00 **Alternative binding proteins: Anticalins** *Arne Skerra*

10.00-10.30 **Coffee break - Posters and Exhibition**

10.30-12.00 **ESTP AGM**

12.00-12.30 **Lunch - Posters and Exhibition**

12.45-13.15 **AiraMatrix Sponsored sessions: GLP- Compliant Digital Peer Review & AI-Based Hepatotoxicity Assessment** *Shahnaz Akhtar, Tijo Thomas & Uttara Joshi*

Session: Targeted protein degraders *Chairs: Kuno Würsch & Robert Kreutzer*

13.15-13.45 **The current & future landscape of molecular degraders of disease-causing proteins** *Ingo Hartung*

13.45-14.15 **Differentiation and Dose Optimization of Aiolos/Ikaros Degrading CELMoD Compounds in Multiple Myeloma** *Michael Amatangelo*

14.15-15.00 **Peripheral neuropathy of Targeted Protein Degraders: Evaluation in preclinical studies and clinical relevance** *Daher Ibrahim Abio*

15.00-15.45 **Coffee break - Posters**

15.45-16.15 **Award ceremony**

Session: Targeted protein degraders *Chairs: Flavia Pasello Dos Santos & Armelle Grevot*

16.15-16.45 **Destruction with a purpose: PROTACs in oncology drug discovery (would include SMARCA2 degrading PROTACs)** *Manfred Koegl*

16.45-17.30 **Targeted protein degraders: safety assessment considerations** *Axel Vicart*

17.30-17.50 **Case Presentation: Assessing the dimension behind the HE: novel technologies to complement tissue-based readouts for gene therapy** *Bettina Amberg, Sabrina Kehm & Kerstin Hahn*

19.30-23.00 **Congress dinner - KLARA**

FRIDAY 29 SEPTEMBER 2023 | ESTP CONGRESS

Session: Miscellaneous *Chairs: Xavier Palazzi & Vanessa Schumacher*

08.30-09.15 **Targeting defects in DNA repair in precision oncology** *Sven Rottenberg*

09.15-10.15 **Implementation of the Weight of Evidence Assessment of human carcinogenic risk assessment according to ICHS1B(R1) - presentations and discussion** *Thomas Nolte, Susanne Brendler-Schwaab & John Vahle*

The Chirukandath Gopinath Award (sponsored by BSTP) will be awarded to Thomas Nolte

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

Session: Miscellaneous *Chairs: Thierry Flandre & Sabrina Schroeder*

10.45-11.15 **Impuritites in Bx and Gene therapies, safety perspective** *Helen Booler & Thierry Flandre*

11.15-11.45 **Immunogenicity of AAV-based gene therapy and new generation biologics** *Fraser McBlane & Hannah Morgan*

11.45-12.15 **Pathology readouts of complex in vitro models in safety assessments** *Nadine Stokar*

12.15-12.55 **Case Presentations:**

12.15-12.35 **Direct Brain Delivery - Thinking Outside the Box** *Deepa Rao*

12.35-12.55 **Healthy appearing cynomolgus monkeys presented gross lesions suggesting a generalized granulomatous disease. All previously performed testing (PCR, Mantoux) revealed negative results. What went wrong?** *Klaus Weber*

12.55-13.10 **Closing Ceremony**

13.10-14.00 **Networking Lunch**

SIDE MEETINGS

Monday 25 September
ESTP Board Meeting
On invitation only
Chair: Silvia Guionaud
Room: Frankfurt
Time: 15.00-18.00

Tuesday 26 September
ESTP digpath committee
Informal gathering
Chair: Lise Bertrand
Room: Frankfurt
Time: 15.30-16.00

Wednesday 27 September
Early Career Meeting
Open meeting
Chair: Simone Tangermann
Room: Frankfurt
Time: 11.35-12.35

Thursday 28 September
ESTP Pathology 2.0 Committee
Chairs: Dirk Schaudien & Josep Monné
Room: Frankfurt
Time: 15.00-15.45

SOCIAL PROGRAM

Tuesday 26 September 2023

18.00-19.15 **Welcome Reception**

Bar Rouge, Messeplatz 10, 4058 Basel

Located in the same building as the Hyperion Hotel

The welcome reception will take place at Bar Rouge in Basel. Bar Rouge offers spectacular panoramic views over the city. As one of the most beautiful rooftop bars in Switzerland and one of the best bars in Basel, Bar Rouge offers a unique ambience that brings you a little closer to heaven.

Registration fee: Included in the registration fee, but registration is required.

The Welcome Reception is only made possible by the generous support of:

Thursday 28 September 2023

19.30-23.00 **Congress Dinner and Dance**

KLARA, Clarastrasse 13, 4058 Basel

The congress dinner & dance will take place at KLARA, only a few minutes by foot from the Hyperion Hotel. KLARA offers a unique but casual dining experience, so no need to dress up for this evening but bring your casuals to dance the night away on the dance floor after dinner. The dining concept offers food stalls from all corners of the world, providing options for everyones liking. Registration fee Included in the registration fee, but registration is required.

TUESDAY 26 SEPTEMBER 2023

SPEAKER ABSTRACTS | IATP SYMPOSIUM

- MEDICAL DEVICE TOXICOLOGY: MODERN DAY SAFETY EVALUATION FOR THE POTENTIAL BIOLOGICAL RISKS ASSOCIATED WITH MEDICAL DEVICES
- CHALLENGES IN BIOCOMPATIBILITY AND REGENERATIVE MEDICINE: FOCUS ON BONE IMPLANT PATHOLOGY - A WORKUP, SHORTFALLS AND FORECASTS
- EXTRACTABLE AND LEACHABLE STUDY FOR MEDICAL DEVICES & THEIR TOXICOLOGICAL ASSESSMENT
- REGULATORY REQUIREMENTS FOR MEDICAL DEVICES REGARDING TOXICOLOGICAL PROPERTIES

SPEAKER ABSTRACTS | ESTP CONGRESS

- KEYNOTE LECTURE: BUILDING THE MEDICINE TOOLBOX: HOW DID WE GET HERE AND WHAT IS THE NEXT BIG THING?
- THE CURRENT STATE OF NUCLEIC ACID THERAPEUTICS
- PATHOLOGY FINDINGS ASSOCIATED WITH SYSTEMIC AND IT DELIVERED ANTI-SENSE OLIGONUCLEOTIDES (ASOS) IN PRECLINICAL SAFETY STUDIES
- ASO-RELATED MORPHOLOGIC FINDINGS AND CHANGES ASSOCIATED WITH INTRATHECAL ADMINISTRATION (IT) OF DRUGS IN NONHUMAN PRIMATES (NHPS) AND RODENTS
- REGULATORY ASPECTS OF PRECLINICAL OLIGONUCLEOTIDE DEVELOPMENT

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08.40-09.30 **Medical Device Toxicology: Modern Day Safety Evaluation for the Potential Biological Risks Associated with Medical Devices**

Nicole Soucy
Boston Scientific, St. Paul, United States

Medical devices represent a complex category of medicinal products with varying definitions depending on which regulatory agency has jurisdiction. In the context of devices used on or in the human body, a common aspect of these definitions is that a medical device is generally intended to be used for specific medicinal purpose where the primary intended action of the device is not achieved through pharmacologic (or other chemical) means. While the regional regulatory frameworks for medical devices are different than for pharmaceutical or biological products, medical device manufacturers are required to evaluate the safety and performance of these products in the context of their intended use. In general, the safety of a medical device is established through a rigorous process of balancing the benefits of the device for its intended use against the potential risks of such use.

As medical device regulations began proliferating in the 1970's, there was a need to establish a harmonized means for determining if these devices, and the materials they are manufactured from, were safe. The Tripartite Biocompatibility Guidance, an agreement between the US, the UK, and Canada and which borrowed heavily from methods established by the US Pharmacopea for the classification of plastics used in pharmaceutical container closure systems, was the first attempt at harmonization. This framework formed the skeleton of the first edition of ISO 10993-1 published in 1992, which is the internationally harmonized basis on which medical device safety is established. While this series of standards has evolved over time, the actual test methods themselves largely remain unchanged from these origins.

Historically, an evaluation of 'biocompatibility' has been a key aspect of medical device safety evaluation and is defined in ISO 10993-1 as "ability of a medical device or material to perform with an appropriate host response in a specific application". The limitation of this definition is that it largely means that a material should have the ability to reside in the body, often for a long period of time, with only low levels of foreign body response. Modern day medical device safety evaluation involves a comprehensive evaluation of the potential adverse effects of the device, inclusive of its materials of construction and manufacture and an assessment of the overall patient safety risk related to the intended use of the device. This assessment is more appropriately aligned with the definition of toxicology; medical device toxicology is therefore a more appropriate description of the overall biological safety evaluation process for these products.

Given that medical device regulatory frameworks are largely based on principles of risk management, the biological evaluation of a medical device follows a tiered approach based on where and for how long the device is used. This is referred to as the device categorization and is driven by both the nature and duration of contact. The nature of contact is categorized based on where the device interfaces with the body, such as intact skin, breached or compromised surfaces, externally communicating, or implantation in tissue, bone, or in contact with blood. For each of

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these categories, there is further sub-categorization based on the duration of device contact across three durations: < 24 hours, < 30 days, or > 30 days. As the nature of contact increases from lower to higher risk, and as the duration of contact increases from shorter to longer time frames, the biological endpoints requiring evaluation also increase. As an example, the biological endpoints relevant for a device implanted in the body, having contact with blood for greater than 30 days would include: cytotoxicity, irritation, sensitization, material mediated pyrogenicity, implantation effects, hemocompatibility, an evaluation of systemic toxicity (acute, subchronic, and chronic), genotoxicity, and carcinogenicity. Historically, the list of endpoints relevant for biological evaluation largely represented a 'checklist of tests' to be conducted. Since 2009 however, these represent endpoints which must be evaluated, often through a combination of information and testing.

The information relevant to this overall evaluation begins with characterization of the physical, morphological, and chemical characteristics of the device. It is also important to understand where in the anatomy the device will be placed, how many devices would be used in a single patient, and if there is anything specific about the patient population that makes them particularly sensitive (e.g. neonate or child). With this information in hand, the assessor identifies the potential biological hazards associated with the substances, estimates the potential biological risks as assessed through scientific rationale and / or testing, and finally determine the overall acceptability of these risks as they related to the intended clinical use of the device. As objective evidence, this evaluation is documented with overall biological safety conclusions for each medical device. Within the risk management framework, this conclusion must take into consideration the risk profile of the device balanced against the intended clinical benefit of that device.

In recent years, there has been a strong interest in refining, reducing, and replacing animal tests for the evaluation of medical devices. Ex vivo and in vitro models have been or are actively being validated for the direct replacement of certain tests, refinement of existing preclinical studies in large animal models to address additional biological endpoints, and development of analytical extractables methods to allow for evaluation of toxicological risks related to potential device leachables are all commonly used in modern medical device safety evaluation.

This presentation will delve into each of these aspects and provide the audience with an in-depth understanding of medical device toxicology today.

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09.30-10.20 **Challenges in Biocompatibility and Regenerative Medicine: Focus on Bone Implant Pathology - a Workup, Shortfalls and Forecasts**

Antoine Alves
NAMSA, Lyon, France

After blood transfusion, bone grafting is the second most frequently performed tissue transplantation in human medical practice. Grafts/substitutes are used to restore, augment, and rehabilitate injured bone. Several factors influence the success of bone implants. Regulatory authorities and clinical practitioners require a burden of proof supported by pre-clinical evidence. Pathologists play an essential role in assessing innovative and emerging bone therapies, but they face numerous challenges. Understanding the various material properties, the mechanical and biological laws interplaying at the material interface, and other technical challenges such as tissues preparation and the place of imaging can help in better evaluating the implant safety, in accordance with international standards. To this end, quantitative pathology using image analysis systems contributes to the objective evaluation of implant performance. These computerized systems are more and more sophisticated - driven by the increasingly use of modern tools such as artificial intelligence and machine learning algorithms. This talk aims to share an experience in understanding the mechanisms governing bone healing in presence of an implant, implications of material properties, and the place of new evaluation tools in medical device pathology. The presentation will address some shortfalls as well, and make forecasts for the further development of regenerative and bone implant pathology studies in upcoming years.

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10.20-10.50 **Extractable and Leachable Study for Medical Devices & their Toxicological Assessment**

Flora Wegener
Fresenius Kabi Bad, Homburg, Germany

To ensure patient safety, precise knowledge about potential contamination associated with medical device materials is critical. For this reason, a robust physico and/or chemical characterization of the medical device is the first and mandatory step of any biocompatibility study performed according to the most recent version of the ISO 10993-1:2018 guidance. The extent of the analysis is strongly dependent on the available knowledge of the device including material characterization, non-clinical and clinical safety data, toxicological data as well as information on possible residual process aids or additive used in the frame of manufacturing process. With increasing complexity of medical devices and combination products, the risk of leachable being introduced into the body increases significantly. Therefore, the design of extractable/leachable study should attentively consider the type/class of device, application scenario and contact time. This complexity required a more defined framework to allow identification of potential biological hazard, estimation, and control of biological risk potentially deriving from the constituents of the medical device. This framework has been provided within the recent update of ISO 10993-18:2020. The standard gives a sound guidance on how extractable/leachable study should be built to generate a comprehensive understanding of the related risks. First step to consider is the determination of the Analytical Evaluation Threshold (AET), specifically introduced in the ISO 10993-18:2020, which convert a dose-based threshold (TTC (Threshold of Toxicological Concern)) to a concentration-based threshold (AET). Extractable/leachables which are below the AET should not be identify, quantify, or reported, since are considered as having an acceptable safety risk without further toxicological assessment. On the contrary, values of extractable/leachable above the AET need to be toxicological assessed according to ISO 10993-17:2002 requirements. Following steps include the selection of extraction condition (i.e., simulated, exaggerated, exhaustive), extraction vehicles (i.e., polar, mid-polar, not polar), and adequate analytical methods (i.e., gas chromatography/mass spectrometry, liquid chromatography/mass spectrometry, inductive coupled plasma- optical emission spectrometry). Selection of these various parameters/conditions will depend on the risk associated with the medical device category based on type, duration of contact and clinical use. Although the strategy described in the ISO 10993-18:2020 on how to conduct and extractable/leachable study seems to be quite straightforward, it should be remembered that medical devices are “not easy” and difficulties could be encountered either during the conduction/plan of the chemical analysis or the toxicological assessment i.e., compatibility of extraction solvent with device material, mismatch between AET calculated and effective limit of detection of the analytical method, higher amount of unidentified compounds. Finally, the appropriate weight should be given to the extractable/leachable results considering them within the complete biocompatibility assessment of the medical device. Therefore, the “how” and “why”, when planning an extractable/leachable study, should always be clearly stated and a tight cooperation between analytical laboratory and qualified toxicologist should be established in order to develop meaningful data. Case studies will be provided and discussed.

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11.20-11.50 **Regulatory Requirements for Medical Devices regarding Toxicological properties**

Uta Bussmeyer
Merz Aesthetics GmbH, Frankfurt, Germany

Medical devices are - in short - instruments, apparatuses, appliances, software, implants, reagents or materials that can be used for medical purposes of, among other things, diagnosis, prevention, monitoring, prediction, prognosis, treatment or alleviation of disease (non-exhaustive definition). They do not achieve their principal intended action by pharmacological, immunological or metabolic means, in or on the human body, but may be assisted in their function by such means. Although medical devices do not have a pharmacological function there are various toxicological aspects to be considered when seeking regulatory approval for medical devices. The main legal act laying down the requirements for medical devices in Europe is the European Medical Device Regulation (MDR) / Regulation (EU) 2017/745. It entered into force on 25 May 2017 with 26 May 2021 as date of application and replaced the Medical Device Directive (MDD) and the Active Implantable Medical Devices Directive (AIMDD). The “General Safety and Performance Requirements” (GSPR) of the MDR require manufacturers to take toxicological aspects of medical devices into consideration early in the development phase when making choices on device design, specifically regarding materials and substances to be used. Devices, which are invasive and come into direct contact with the human body or (re)administer medicines, body liquids or other substances, or transport the latter underly specific requirements regarding CMR (carcinogenic, mutagenic or toxic to reproduction) substances or agents with endocrine-disrupting properties. Particular requirements have been introduced for devices that are composed of substances or combinations of substances that are intended to be introduced into the human body and that are absorbed by or locally dispersed in the human body. Toxicological data including ADME (absorption, distribution, metabolism and excretion), possible interactions of those substances, their metabolism in the human body, their local tolerance and toxicity are required to achieve regulatory approval of such devices. Data on single-dose toxicity, repeat-dose toxicity, genotoxicity, carcinogenicity as well as reproductive and developmental toxicity may have to be provided to obtain the desired product certificate. In summary, the introduction of MDR increased the importance of toxicology for medical device certification.

TUESDAY 26 SEPTEMBER 2023 | ESTP CONGRESS

13.45-14.45 **Keynote Lecture: Building the medicine toolbox: how did we get here and what is the Next Big Thing?**

Richard Haworth
AstraZeneca, Cambridge, United Kingdom

It's an exciting time to be working in BioPharma R&D! Scientific advances continue to provide new ways to intervene in human biology and tackle drug targets beyond the reach of small molecules and conventional monoclonal antibodies. This talk will look backwards to understand why and how new drug modalities were developed. It will look forwards to speculate on what opportunities lie ahead. For the preclinical safety scientist and toxicologic pathologist, the fundamental challenge of predicting human therapeutic index from nonclinical safety and efficacy data remains relevant. This applies irrespective of whether a project team selects an antisense oligonucleotide, a protac or a cell therapy for their new target. We generate data and make judgements at a time when knowledge of clinical safety for new modalities and delivery systems is often limited or absent. To improve the probability of a project delivering a new medicine and delivering patient benefit, we need to continue to increase our fundamental understanding of disease biology and to increase the human relevance of our safety and efficacy models. This talk will consider how single cell transcriptomics, use of microphysiological systems, proteomics and multimodal imaging are helping with this task. Plenty of scientific opportunities for a curious mind!

14.45-15.30 **The current state of nucleic acid therapeutics**

Roy van der Meel
Eindhoven University of Technology, Eindhoven, Netherlands

Nucleic acid therapeutics are revolutionizing healthcare via gene inhibition, addition, replacement or editing. However, nucleic acid-based drugs require chemical modifications and sophisticated platform nanotechnologies to prevent their degradation, reduce immunostimulatory effects, and ensure intracellular delivery[1]. While there are several viral vector-based genetic drugs approved by the FDA and EMA for *ex vivo* and *in vivo* approaches, this talk will focus on non-viral platforms enabling nucleic acid therapeutics following systemic administration.
These include chemically modified antisense oligonucleotides, N-acetylgalactosamine (GalNAc) ligand-modified siRNA conjugates, and lipid nanoparticles (LNPs) containing siRNA or mRNA. Over the last 25 years, these platforms have enabled the approval of 18 nucleic acid-based drugs with several more undergoing late-stage clinical evaluation[2]. For each platform, I will briefly explain the mode of action, highlight key technological aspects that have facilitated its clinical translation, and discuss its therapeutic effectiveness and adverse reactions. Finally, I will address how these platform technologies are enabling next generation nucleic acid therapeutics such as gene editing approaches.

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16.15-16.45 **Pathology findings associated with systemic and IT delivered Anti-Sense Oligonucleotides (ASOs) in preclinical safety studies**

Kuldeep Singh
Wave Life Sciences, Lexington, United States

Systemic effects of ASOs include immune stimulation, complement system activation, clotting time prolongation, thrombocytopenia, injection site reaction, and organ toxicities. Common histopathology findings include dose- and duration-dependent infiltration of vacuolated macrophages and mononuclear inflammatory cells (could be non-dose-dependent) in multiple organs with systemic dosing, and predominantly in CNS with IT dosing. Dose-limiting toxicologic findings are often species-related, observed in the liver and kidneys, and are independent of chemistry and class. A functional impact is observed on the hepatic and renal systems with correlative changes in clinical pathology parameters at high doses. Mechanisms for such toxicities will be discussed along with the clinical relevance and use of predictive assays to screen such liabilities.

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16.45-17.15 **ASO-related morphologic findings and changes associated with intrathecal administration (IT) of drugs in nonhuman primates (NHPs) and rodents**

Annette Romeike
Labcorp Early Development Services GmbH, Münster, Germany

Over the last decade, the development of ASOs has steadily increased, particularly for the treatment of nervous system disorders, and represents a large proportion of the modalities developed at the Labcorp Early Development Services Primate Center in Münster (Germany). With intrathecal (IT) administration of ASOs in NHPs or rodents into the cerebrospinal fluid to achieve spinal cord and brain tissue concentrations sufficient to elicit the intended therapeutic effect, the toxicologic pathologist might face challenges to discriminate between test-article, procedure-related or spontaneous findings. Examples of such cases in NHPs and rodents will be given.

17.15-18.00 **Regulatory aspects of preclinical oligonucleotide development**

Helen-Marie Dunmore
Charles River, Ilkley, United Kingdom

Antisense oligonucleotides (ASOs), short interfering RNAs (siRNAs) and aptamers are oligonucleotide-based pharmaceuticals. Antisense oligonucleotides are short, single-stranded oligonucleotides that typically consist of 13-25 nucleotides, that bind to their complimentary mRNA by Watson-Crick based pairing.1 siRNAs are double-stranded RNAs, that usually consist of around 20 nucleotides. Aptamers are single-stranded oligonucleotides that usually consist of 15-45 nucleotides. Oligonucleotide-based pharmaceuticals have a promising role in treating genetic diseases for which there are currently no or limited treatment options. To date, a number of oligonucleotide-based therapeutics are in nonclinical or clinical development and some have achieved licensing status. As oligonucleotide-based drugs share properties with both chemical and biological pharmaceuticals, this can pose challenges in relation to the nonclinical regulatory pathway. Whilst focus will be on the traditional nonclinical strategy for this class of compounds, the presentation will also touch on the nonclinical research pathway for the testing of individualised products for severely debilitating or life-threatening diseases for non-commercial purposes in the US.

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08.30-09.15 **mRNA platform approach for the preclinical development of vaccines**

Shambhunath Choudhary
Pfizer Pearl River, Newyork, United States

The emergence of SARS-CoV-2 at the end of 2019 required the swift development of a vaccine to address the pandemic. To assess the local tolerance, systemic toxicity, and immune response to mRNA vaccine candidates encoding immunogens that were derived from the spike (S) glycoprotein of SARS-CoV-2 encapsulated in lipid nanoparticles (LNPs), nonclinical GLP-compliant studies were initiated in Wistar Han rats. Vaccine candidates were administered intramuscularly once weekly for three doses at 30 and/or 100 µg followed by a 3-week recovery period. The main microscopic findings included increased cellularity in the draining lymph nodes, spleen, and bone marrow, acute inflammation and edema at the injection site, and hepatocellular vacuolation in the liver; these microscopic findings correlated with macroscopic observations of enlarged lymph nodes/spleen and thickened and/or dark injection sites as well as clinical pathology findings of higher white blood cell counts and acute phase reactant concentrations. These findings were generally attributed to the anticipated immune and inflammatory responses to the vaccines. These studies demonstrated safety and tolerability in rats, supporting SARS-CoV-2 mRNA-LNP vaccine clinical development. The nonclinical findings were comparable between the different vaccine candidates at the same dose level, and evidence of a dose effect on nonclinical findings and serology were noted. The high reproducibility of observations made in two independent GLP toxicity studies using the same platform, but different encoding antigens demonstrate that a platform toxicity approach would be suitable for the development of an mRNA-LNP vaccine.

09.15-10.00 **Next generation bispecific antibodies and antibody fusion proteins for cancer immunotherapy**

Christian Klein
Roche Glycart AG, Schlieren, Switzerland

In the past decade much progress has been seen in the field of T cell bispecific antibodies that engage T cells for killing of tumor cells, particularly in the treatment of B cell malignancies and multiple myeloma. Several T cell bispecific antibodies have been approved. In this presentation I will provide an overview of recent development of T cell bispecific antibodies, their application in solid tumors as well as their combination with co-stimulatory bispecific antibodies and antibody fusion proteins. Finally, the concept of a novel PD-1 cis-targeted IL2v immunocytokine will be introduced.

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10.30-11.15 **Biologic Associated Immune-mediated Renal Disorders in nonclinical toxicity studies and their relationship to the clinical syndrome.of BAIRD**

Maronpot Guest Lecture, Sponsored by IATP and the Telikicherla Higher Education Foundation (THE)

Ken Frazier

Private consultant, Alligator Point, FL, United States

As a greater number of alternative classes of medicines such as biotherapeutics, chimeric antigen receptor T-cell (CAR-T) therapies, antibody-drug conjugates, or other immunomodulatory drugs come to market, the presentation of drug-induced nephrotoxicity is changing. Instead of cytotoxic related tubule toxicity which was previously so common with small molecules, potential pathogenic mechanisms related to immunogenicity, immune complex formation, and stimulation of downstream proinflammatory pathways with some of these alternative medicine classes have resulted in the potential for glomerulonephritis, acute interstitial nephritis, renal vasculitis, and other immune-mediated renal disorders in human patients. This contrasts with nonclinical toxicity studies, where biologic therapies more often result in vasculitis and glomerulonephritis associated with antidrug antibodies, which are not always predictive of clinical effects. This lecture will review biologic associated immune-mediated renal disorders in humans and compare and contrast the changes between this BAIRD clinical syndrome and the immune-related conditions associated with biologic and other novel alternative therapy administration in animal safety assessment studies. Strategies for diagnosis and interpretation of clinical risk assessment will be addressed.

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13.15-14.00 **The History And Evolution Of Gene Therapy**

Jonathan Appleby
Cell and Gene Therapy Catapult, London, United Kingdom

After decades of investment and development, Advanced Therapeutic Medicinal Products (ATMPs) are beginning to deliver their long expected medical potential. Despite possessing a somewhat simplistic scientific appeal, progress has been a hard-fought. In this presentation I will introduce some common viral vector platforms and review what we have learnt from key milestones in their development.

14.00-14.45 **The comprehensive pathologist toolbox for the preclinical development of AAV-based gene therapy products**

Basel Assaf
Sanofi, Cambridge, United States

The advancements in sequencing the human genome and understanding the molecular and genetic mechanisms of several diseases have launched the era of genetic medicine as a therapeutic modality for the treatment of several rare diseases and cancers. Despite the progressive adoption of such therapeutic modality, safety assessment remains complex and is designed on a case-by-case basis that is determined by the disease indication and product attributes. Veterinary pathologists are inherently trained and positioned to be influential in comparative medicine and translational sciences, and naturally capable of combining basic scientific knowledge with observations in nonclinical studies and human risk assessment. This talk will focus on providing the pathologists a set of investigative and toxicologic pathology tools and applications focusing on recombinant adeno-associated virus-based GTx to exemplify the main points to be considered and to drive a major impact in the nonclinical research and development of GTx products.

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14.45-15.30 **Liver gene transfer and editing: challenges and perspectives**

Alessio Cantore
San Raffaele Telethon Insitute for Gene Therapy (SR-Tiget), Milano, Italy

The liver is an important target organ for in vivo gene therapy, offering the potential to treat coagulation and metabolic diseases. Liver-directed gene therapy with adeno-associated (AAV) vectors containing a clotting factor transgene has demonstrated very positive results in adult patients with hemophilia. However, since AAV vectors do not actively integrate into the host cell genome, they are diluted following cell division during liver growth, making their use in pediatric patients currently difficult. In contrast, lentiviral (LV) vectors integrate into the chromatin of target cells and are maintained even if the cells divide. We have developed LV that achieve stable expression of the transgene in the liver after systemic administration and allow for dose-dependent reconstitution of coagulation factor IX (FIX) activity in animal models of haemophilia.
We recently generated more LVs resistant to phagocytosis, which, after intravenous (i.v.) administration in non-human primates (NHP), showed selective gene transfer to liver and spleen and particularly enhanced hepatocyte gene transfer, achieving up to 300% of normal activity of a human FIX transgene, with no signs of toxicity. To apply our gene therapy strategy to haemophilia A, we optimized the coagulation factor VIII (FVIII) transgene. After i.v. of these LVs in NHP we observed 60-100% of the normal circulating FVIII concentration. More recently we have applied gene therapy in mouse models of two diseases of hepatic metabolism: familial hypercholesterolemia and methylmalonic acidemia, due to the lack, respectively, of the low-density lipoprotein receptor and of an enzyme involved in the catabolism of some amino acids and fat. In both cases, in vivo gene therapy to the liver with LV allowed to obtain a stable nearly life-long correction of the disease phenotype, even following neonatal administration.
Since gene transfer with integrating vectors remains associated with imperfect control of transgene expression and the risk of insertional mutagenesis, we are also exploring in vivo gene editing as a therapeutic strategy and compare advanced LV-mediated gene delivery and site-specific nuclease mediated gene editing strategies in terms of feasibility, efficiency, therapeutic efficacy, safety and durability in vitro and in vivo. Targeted integration of a partial corrective cDNA into the endogenous locus may be particularly important in the context of cell-autonomous metabolic diseases, to reconstitute natural expression and regulation of the corrective cDNA. During this presentation, I will present and discuss advantages, disadvantages and safety concerns of gene transfer and gene editing to the liver.
Collectively liver-directed gene therapies promise to become novel therapeutic approches with the potential to revoluzionise medicine.

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16.00-16.30 **General biopharmaceutical/regulatory/clinical aspects of Gene Therapies including rAAV vectors**

Andreas Hartmann
Novartis Pharma AG, Basel, Switzerland

Gene therapies are being developed to become important treatment option for patients. Understanding genetic contributions to disease, the scientific basis of gene therapy has become an interesting approach, in particular for monogenic diseases where a gene defect can be clearly linked as causative for a disease.
While the number of clinical studies with experimental gene therapies has increased significantly over the past several years, there are certain challenges for biomedical researchers how to design, manufacture and deliver corrective genes to treat disease. Cell therapies developed from genetic modifications in vitro and in vivo gene delivery therapies demonstrate that challenges can be overcome, and impressive clinical benefits can be achieved. However, to date, a comparatively low number (approximately 20) of FDA-approved cell and gene therapy products are available. First, an important challenge so far has been how to effectively deliver synthetic genetic therapies to target tissues and cells. Second, to be able to make a product available to larger patient populations and eventually marketing of a product, the process of manufacturing a consistent and well characterized drug product needs to be established meeting regulatory requirements and expectations for a consistent drug product.
The presentation will focus on key issues arising from non-clinical to clinical development; provide examples for safety aspects, mainly on recombinant AAV vectors and the establishment of clinical safety biomarkers and monitoring; outline current regulatory frameworks and avenues to interactions with regulators; and address some questions regarding non-clinical to clinical PK/PD extrapolations, including determination of clinical dose levels based on animal models.
The presentation will focus on key issues arising from non-clinical to clinical development. These include issues reaching target cells and tissues; amount of transgene expression required for stable transfection/transduction; adequacy of animal models of the target disease; questions on target patients populations; pre-existing and induced immunity to the vector and transgene; potential consequences of host genomic integration and/or germline transmission. Furthermore, examples for safety aspects will be discussed, mainly focused on recombinant AAV vectors and the establishment of clinical safety biomarkers and monitoring. Questions regarding non-clinical to clinical PK/PD extrapolations, including determination of clinical dose levels based on animal models will be discussed.
Since recombinant AAV-based vectors bear similarities, examples for an approach to utilize platform-based data instead of individual data will be presented. By utilizing existing data across different vectors, animal use may be reduced.
Regarding the current regulatory environment, regulatory frameworks and avenues for interactions with regulators will be presented. At present, regional guidance on non-clinical safety is high-level and conceptual and often, no specific requirements on key questions exist. Critical aspects of a non-clinical program to support clinical development and marketing will be highlighted.

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16.30-17.15

Development of PLX-R18 Cell Therapy as a Countermeasure for Hematopoietic Acute Radiation Syndrome

Marianna Truman [1], Gilad Kunis [1], Christie Orschell [2], Sanchita Ghosh [3], Vidya Kumar [3], Michal Sheleg [1], Nitsan Halevy [1], Racheli Ofir [4], Arthur Machlenkin [1]

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Pluri Biotech is developing a novel PLX-R18 cell therapy for the treatment of Hematopoietic Acute Radiation Syndrome (H-ARS) in subjects that were exposed or are suspected to have been exposed to high dose ionizing radiation.

PLX-R18 is a novel cell-based medicinal product, comprised of human placenta derived stromal cells delivered through intramuscular (*IM*) injection. The living cells adaptively secrete a cocktail of active hematopoietic factors. These factors, thoroughly characterized at mRNA and protein levels, act together to produce optimal therapeutic efficacy by facilitating the recovery of hematopoietic progenitor cells in the bone marrow and the regeneration of multiple blood lineage cell counts in the peripheral blood. To decipher the molecular mechanisms underlying PLX-R18 activity, individual genes that are known to affect the hematopoietic system were silenced using CRISPR/Cas9 and the effect of the modified PLX-R18 cells on hematopoiesis was examined.

Pre-clinical studies demonstrated that PLX-R18 administered (1st dose at 24 h after and 2nd dose 5 days after exposure) to animals exposed to H-ARS targeted dose of radiation, significantly increased survival rates from 29% in the control group to up to 97% in the PLX-R18 treated group ($p<0.001$). Studies conducted at the Armed Forces Radiobiology Research Institute (U.S. Department of Defense Institution) have shown that PLX-R18, administered as a prophylactic measure with 1st dose at 24 hours before radiation exposure and 2nd dose at 72 hours after exposure, resulted in a significant increase in survival rates, from 4% survival rate in the placebo group to 74% in the PLX-R18 treated group (log-rank test $p< 0.0001$). In addition, the data show a significant increase in recovery of white blood cells ($p = 0.0047$), platelets ($p = 0.0070$), neutrophils ($p = 0.0003$) and lymphocytes ($p = 0.0025$) counts compared to vehicle control, and a favorable safety profile.

Additionally, PLX-R18 was tested in humans with incomplete hematopoietic recovery following Hematopoietic Cell Transplantation (HCT) and was well tolerated with a favorable safety profile. Patients treated with PLX-R18 showed an increase in all three blood cell lineages compared to the baseline with platelets ($p<0.001$), hemoglobin ($p=0.02$) and neutrophils ($p=0.15$) levels increasing, as early as 1 month following PLX-R18 administration and enduring up to 12 months following treatment. Furthemore, PLX-R18 treatment result in a significant reduction in the mean number of transfused units from a monthly rate of 5.09 to monthly rate to 0.55 for platelets ($p=0.045$) and 2.91 to 0 for red blood cells ($p=0.0005$) over 12 months of follow-up.

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Recent geo-political events have reinforced the need for the global community to better prepare for nuclear disasters, such as seeking new medical countermeasures that are more cost-efficient and scalable than current options.
Disclaimer: The opinions and assertions expressed herein are those of the presenter and do not necessarily reflect the official policy or position of the Uniformed Services University or the Department of Defense.
The following authors (CMO, SPG and VPK) declare no conflict of interest. Rest of the authors are are employees of Pluri Biotech or are associated with the company and may stand to benefit financially from the successful development of the compound.

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17.15-18.00 **Gene-modified differentiated lymphocytes (cell therapy)**

Jan Klapwijk
Cornelis Consulting Ltd, Ware, United Kingdom

In this presentation I will briefly review lymphocyte pathobiology before providing the rationale for the use of gene-modification of different sub-types of lymphocytes to enhance their utility in various disease settings. Ways in which these cells can be modified (particularly via ex vivo gene therapy) will be explained. A major focus will be on safety issues, including those which are somewhat inherent to particular therapies eg cytokine release syndrome following CAR-T (effector) cell therapy, as well as those which are more theoretical / less likely (eg insertional mutagenesis). Proposals for attempting to address these non-clinically will be discussed. The presentation will also indicate products where clinical successes (including product registrations) have been seen, as well as products at earlier stages / facing particular challenges.

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08.30-09.15 **ADCs - still emerging or coming of age?**

Kuno Würsch
Novartis AG, Basel, Switzerland

Development of antibody drug conjugates (ADC) has started decades ago and at the time it was believed to finally yield the significant improvement of the therapeutic index for some of the efficacious but toxic chemotherapies. It was anticipated that vectorization of the payload by conjugating it to a mAb would allow for a more targeted drug delivery. The sobering reality is though that currently approved as well as many in-development ADCs have so far not significantly reduced toxicity and their therapeutic index remain small (Colombo and Rich, 2022). This presentation explores some of the reasons why the initial generations of ADCs employing cytotoxic payloads may have fallen short of expectations and what lessons can be learned for a future generation of ADCs employing more advanced design features as well as different payloads. ADCs conjugated with cytotoxic payloads usually demonstrate a similar toxicity profile as the payload, but some important variations exist and although not always fully understood, these can provide important learnings. The antibody target (epitope) has been the focus and starting point of most ADC programs with more limited considerations for the payload and other ADC design features such as drug-antibody-ratio (DAR) of Fc modifications. An inverse development strategy starting with tumor-specific payloads may address some of the current liabilities. This presentation will include an example of an ADC development program that has started by selecting a tumor-specific payload. Data will be presented highlighting the in vitro proof of concept studies and in vivo validation of this strategy to potentially improve the therapeutic index.

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09.15-10.00 **Alternative binding proteins: Anticalins**

Arne Skerra
Technical University of Munich, Freising, Germany

Anticalin® proteins are an emerging class of clinical-stage biopharmaceuticals with high potential as an alternative to antibodies. Anticalins are generated by combinatorial design from natural lipocalins, which are abundant plasma proteins in humans and also found in many other organisms, usually serving for the delivery or storage of vitamins, hormones, and metabolites or as inhibitors. The lipocalins exhibit a simple, compact fold dominated by a central β-barrel, with an α-helix attached to its side, which supports four structurally variable loops at one end. These four loops form the entrance to a ligand pocket, thus showing similarity to the six CDRs of an antibody that shape its antigen-binding site. However, contrasting to immunoglobulins, lipocalins have a much smaller size (160-180 residues), they are composed of a single polypeptide chain and they can be produced at high yields in microbial expression systems. Reshaping of the variable loop region of lipocalins using protein design techniques results in so-called Anticalins which can recognize and tightly bind a wide range of medically relevant targets. In fact, combinatorial gene libraries together with powerful molecular selection techniques, such as phage display or bacterial surface display, have enabled the expansion of the natural ligand specificities of lipocalins from predominantly small molecules to peptides and proteins. This biomolecular concept has been validated by structural analyses of a series of Anticalin/target complexes, which confirm the mechanistic analogy to the recognition of antigens by immunoglobulins. Promising Anticalin lead candidates have reached different preclinical and clinical development stages, up to phase II, in the areas of (immuno)oncology, metabolic as well as respiratory diseases, as antidotes to treat intoxications and as novel antibiotics. Antagonistic Anticalin drug conjugates have been developed not only for systemic administration but also for intravitreal injection into the eye and for inhaled delivery directly to the lungs. Furthermore, the robust format and small size of Anticalin proteins allows for modification in several ways, both as fusion proteins - including bispecific Anticalin-antibody fusion proteins – and by chemical coupling, for example, to generate radionuclide-chelate or toxin conjugates or to prolong plasma half-life. Thus, Anticalins represent more than a surrogate of conventional antibodies, offering promising and potentially superior features as next-generation biologics.

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13.15-13.45 **The current & future landscape of molecular degraders of disease-causing proteins**

Ingo Hartung
Merck Healthcare KGaA, Darmstadt, Germany

Degrading disease-causing proteins with small molecules has the potential to significantly change the clinical drug modality landscape. Small molecule protein degraders allow to directly recapitulate genetic knock-down findings with an easy to apply, titratable & reversibly acting drug modality. In my presentation I will provide an overview about the clinical status of the two most important molecular degrader subclasses: molecular glue degraders and bifunctional proteolysis-targeting chimeras (PROTACs). I am going to discuss the target space which is addressed with the current generation of degrader molecules and most significant clinical findings. I am going to highlight the state-of-the art of rationally discovering such degraders and key challenges for their preclinical assessment.

13.45-14.15 **Differentiation and Dose Optimization of Aiolos/Ikaros Degrading CELMoD Compounds in Multiple Myeloma**

Michael Amatangelo
Bristol Myers Squibb, Summit, NJ, United States

Cereblon E3 ligase modulators (CELMoD) are a class of molecular glue protein degraders that co-opt cereblon, the adaptor protein for the cullin-RING ubiquitin E3 ligase, to induce ubiquitination and degradation of target protein substrates. The immunomodulatory imide (IMiD) agents, lenalidomide (LEN) and pomalidomide (POM), are CELMoD agents that have been the foundation of treatment for multiple myeloma over the last decade. Iberdomide (IBER; CC-220) and Mezigdomide (MEZI; CC-92480) are novel, highly potent CELMoD agents selected for development based on learnings of the LEN and POM mechanism of action to induce rapid and maximal degradation of Ikaros and Aiolos, key transcription factors for hematopoietic cell development/differentiation and myeloma cell survival.
IBER and MEZI possess superior potency vs IMiD agents due to their unique cereblon-binding interactions, which induce more efficient allosteric rearrangement of the cereblon-substrate binding site to an active/closed conformation promoting maximal substrate-binding capacity. While POM (at saturating concentrations) only induces approximately 20% of cereblon molecules into a closed/active state, IBER and MEZI promote approximately 50% and 100% of cereblon molecules into a closed/active state, respectively. These enhanced binding characteristics of IBER and MEZI result in faster kinetics of substrate degradation, increased cytotoxic effects in myeloma cells and enhanced immune stimulation compared to IMiD agents as well as activity in preclinical models of LEN and POM resistance. In addition, unlike LEN and POM, IBER and MEZI are administered as a single enantiomer, the S-isomer and are relatively resistant to racemization. This unique chiral structure increases exposure to the enantiomer with higher cereblon binding affinity and may lead to lower rates of sedation/fatigue in the clinic, which have been attributed to

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the R-isomer of thalidomide. Given these properties, IBER and MEZI are currently being evaluated for treatment of multiple myeloma.
With the aim of clinically differentiating IBER and MEZI and informing dose and schedule, a clinical biomarker strategy was developed focused on learnings from preclinical pharmacology studies and was implemented in the phase 1/2 clinical studies for IBER and MEZI, CC-220-MM-001 (NCT02773030), CC-92480-MM-001 (NCT03374085) and CC-92480-MM-002 (NCT03989414). These included assessments of substrate degradation, pharmacodynamic effects on immune cell populations, (B, T and NK cells), changes in serum free light chains (tumor burden biomarker), and changes in absolute neutrophil counts (as a surrogate for treatment tolerability). Dose, exposure, and schedule dependent changes were observed for the above biomarkers, and several appeared to saturate at higher doses. IBER or MEZI treatment resulted in potent Aiolos/Ikaros degradation in subjects at all doses tested. MEZI showed more efficient substrate degradation corresponding to rapid decreases in serum free light chain with greater decreases in absolute neutrophil counts, while IBER demonstrated more limited neutropenia. Notably, dosing periods of MEZI for 7 days or less appeared to allow for tumor recovery over the treatment cycle, supporting a dosing schedule longer than 7 days. Conversely, a 7-day dosing holiday allowed for recovery of substates and absolute neuropil counts, supporting a 7-day dosing holiday during each cycle to increase tolerability. Consistent with IBER or MEZI mechanism of action, treatment also resulted in dose dependent decreases in absolute B-cells and increases in T-cell activation that were most prominent with 14 days or more of continuous dosing and saturated at higher doses.
Based on the safety, efficacy, pharmacokinetic and pharmacodynamic results from these studies, IBER dosed for 21 days out of a 28-day schedule at the 1.0, 1.3, and 1.6 mg doses were chosen for dose optimization in the first phase 3 trial for IBER in the relapse/refractory myeloma setting (NCT04975997) and at the 0.75, 1.0, and 1.3 mg doses were selected for dose optimization of IBER monotherapy in the newly diagnosed myeloma maintenance setting (with the goal of increasing IBER tolerability with long-term treatment) (NCT05827016). For MEZI, further exploration of doses 0.3, 0.6 and 1.0 mg on a 14 out of 21-day or 21 out of 28-day schedule were chosen to determine the optimal dose to balance safety and efficacy in phase 3 studies in the relapse/refractory myeloma setting (NCT05519085 and NCT05552976). Together, these results highlight how deep understanding of protein degrader characteristics and substrate biology can be used to inform clinical development.

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14.15-15.00 **Peripheral neuropathy of Targeted Protein Degraders: Evaluation in preclinical studies and clinical relevance**

Daher Ibrahim Aibo
Novartis, Parsippany, United States

Peripheral neuropathy has been observed clinically and morphologically with a subset of cereblon-binding targeted protein degraders in rodent or non-rodent toxicology species. This neuropathy is characterized by peripheral nerve and dorsal root ganglia neurons degeneration and necrosis and neurofilament light chain correlated well with nervous tissues changes. Utilizing some of our preclinical degraders with established peripheral neurotoxicty, we demonstrated that cereblon knockout out mice are protected from this peripheral neuropathy. Marketed cereblon-recruiting immunomodulatory imide drugs also have variable levels of peripheral neurotoxicty indicating a possible cereblon-dependency of this toxicity.

16.15-16.45 **Destruction with a purpose: PROTACs in oncology drug discovery (would include SMARCA2 degrading PROTACs)**

Manfred Koegl
Boeringer Ingelheim RCV GmbH & Co KG, Vienna, Austria

Targeted protein degradation by proteolysis targeting chimeras (PROTACs) is an exciting new option in drug discovery. The loss in protein levels caused by PROTACs makes targets accessible that are not responsive to small molecule inhibition. Moreover, the observed increase in selectivity observed when binders are turned into degraders can be used to selectively target paralogues. In oncology, loss of the chromatin modifier SMARCA2 has been shown to selectively affect tumors that have lost the paralogue SMARCA4. We have developed heterobifunctional degraders that selectively target SMARCA2 using binders to the bromodomain of SMARCA2 linked to binders of the E3 ligase VHL. Despite their size, these compounds could be optimized to oral bioavailability and were shown to curb tumor growth in vivo upon oral administration. I will discuss the novel opportunities that arise with the advent of PROTACs, as well as novel challenges and pitfalls.

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16.45-17.30 **Targeted protein degraders: safety assessment considerations**

Axel Vicart
Novartis Institutes for Biomedical Research, Basel, Switzerland

Targeted protein degradation is one of the most recent and rapidly expanding small-molecule drug modalities that exploits the endogenous ubiquitin proteasome system to degrade a protein of interest for therapeutic benefit. The approach for assessing the safety of targeted protein degraders (TPDs) is consistent with general practices and guiding principles of small-molecule toxicology but their unique mode of action implies new challenges. These include potential exacerbated toxicity related to prolonged primary target degradation and off-target protein degradation. Disturbance of the normal functions of TPD-engaged ubiquitin ligases or more generally of the proteasome are additional TPD-specific theoretical concerns. The selection of appropriate species for toxicology studies is critical since subtle differences in the sequences of the TPD-interacting proteins (ubiquitin ligase as well as the on- and off-targets) can significantly influence TPD activities.
This is exemplified by the no- or lower sensitivity of rodents and rabbits to thalidomide-mediated human teratogenicity. In addition, a subset of degraders have been associated with peripheral neurotoxicity. A range of *in vitro* safety assays combined with specific *in vivo* study endpoints can be deployed for the early safety assessment and de-risking of TPD drug candidates.

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SPEAKER ABSTRACTS | ESTP CONGRESS

- TARGETING DEFECTS IN DNA REPAIR IN PRECISION ONCOLOGY
- IMPLEMENTATION OF THE WEIGHT OF EVIDENCE ASSESSMENT OF HUMAN CARCINOGENIC RISK ASSESSMENT ACCORDING TO ICHS1B(R1) - PRESENTATIONS AND DISCUSSION
- IMPURITITES IN BX AND GENE THERAPIES, SAFETY PERSPECTIVE
- IMMUNOGENICITY OF AAV-BASED GENE THERAPY AND NEW GENERATION BIOLOGICS
- PATHOLOGY READOUTS OF COMPLEX IN VITRO MODELS IN SAFETY ASSESSMENTS

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08.30-09.15 **Targeting defects in DNA repair in precision oncology**

Sven Rottenberg
University of Bern, Bern, Switzerland

Many cancers have a defective DNA damage response (DDR), which underlies the frequently observed genomic instability. Such DDR alterations cause an accumulation of tumorigenic mutations, and a major goal of precision oncology is to exploit them for individualized cancer therapy. A prime example for this therapeutic concept is the vulnerability of BRCA1- or BRCA2-deficient cells to poly(ADP-ribose) polymerase (PARP) inhibition with high clinical response rates, especially in ovarian cancer. By adapting the genetic concept of synthetic lethality to cancer therapy, it has resulted in the registration of several PARP inhibitors (PARPi) in breast (olaparib and talazoparib) and ovarian cancer (olaparib, niraparib and rucaparib. Unfortunately, PARPi is no exception to the rule that drug resistance inevitably follows the introduction of novel anti-cancer therapies in the clinic. Despite significant improvement of patient progression-free survival, PARPi resistance represents a major clinical hurdle. In my talk I will present approaches how we try to understand the precise resistance mechanisms and how the study of drug resistance provides new insights into basic mechanisms of the DDR machinery.

09.15-10.15 **Implementation of the Weight of Evidence Assessment of human carcinogenic risk assessment according to ICHS1B(R1) - presentations and discussion**

The Chirukandath Gopinath Award (sponsored by BSTP) will be awarded to Thomas Nolte

Thomas Nolte [1], Susanne Brendler-Schwaab [2], John Vahle [3]
[1] Boehringer Ingelheim Pharma GmbH & Co. KG; Member, Biberach an der Riss, Germany, [2] Federal Institute for Drugs and Medical Devices (BfArM), Bonn, Germany, [3] Lilly Research Laboratories, Indianapolis, United States

The finalization of the ICHS1B(R1) in August 2022 introduced an alternative and integrative approach of human carcinogenic risk assessment that may be applicable to certain development programs of small molecule pharmaceuticals. An outcome of this assessment could be that a 2-year carcinogenicity study in rats may not add value to human carcinogenicity risk assessment and in consequence may not be needed. Basic principles of this approach will be presented in a condensed form. The major focus will be on aspects of implementation of this new Weight-of-Evidence approach of human carcinogenicity risk assessment from (I) the regulatory and (II) the sponsor perspective. The procedure of regulatory advice in the EU will be presented and a standard package of briefing material as well as the regulatory relevance of a response will be outlined. Sponsor aspects will include the impact on nonclinical development programs, exemplified by real-world experience. This will include aspects of target-related carcinogenic risk assessment, secondary pharmacology screens, a good understanding of the pathology and pathophysiology of toxicity findings of concern and hormonal effects. The contribution of non-standard end points implemented into standard toxicity studies or into dedicated mechanistic studies will be highlighted. Other major aspects will be the impact on timing of the chronic toxicity study in rats and strategies of regulatory interaction in different regions. Interactions between panelists and the audience will be integral part of this panel discussion.

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10.45-11.15 **Impuritites in Bx and Gene therapies, safety perspective**

Helen Booler, Thierry Flandre
Novartis Institutes for BioMedical Research, Basel, Switzerland

Impurities are an important consideration in drug products, and have the potential to impact the products quality, safety, and efficacy. In the case of biologics (recombinant proteins, antibody fragments, mono/bi/tri-specific antibodies) and AAVs, these impurities can include host cell proteins (HCPs), endotoxins, beta glucans defective protein/particles, nucleic acids and/or virus components. Although such impurities are normally controlled and removed during material purification, some may be copurified with the therapeutic drug - impacting tolerability, safety and risk assessment (De Zafra et al 2015, Srivastava et al 2021, Vanderlaan et al 2018). The safety-related risks, findings observed, and the impact of these responses may vary with the type of product, the impurity itself and the route of administration. Here we discuss these aspects and why being aware of their presence is important to understand any potential unexpected pathology findings or high immunogenicity, and provide some examples of the impact of impurities in drug development.

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11.15-11.45 **Immunogenicity of AAV-based gene therapy and new generation biologics**

Hannah Morgan , Fraser McBlane
Novartis Basel Switzerland

Immunogenicity (the development of an adaptive immune response to a biotherapeutic) is a common but, usually, unwanted aspect of all therapeutic modalities. The focus will be on AAV gene therapies and novel antibody formats (e.g. ADCs and multi-specific antibodies).
This talk will focus on two aspects - potential novel mechanisms by which these therapeutic modalities may elicit an immune response and the potential consequences of pre-existing or drug-induced ADA development on safety, efficacy and pharmacokinetics. Additionally, in viral gene therapies, the route of administration and intended site of expression of the transgene product will also be described.
Case studies (real and hypothetical) will highlight these considerations and how they may impact the initial immunogenicity risk assessment as well as toxicology and PK/PD assessments. Finally, potential mitigation strategies will be discussed.

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11.45-12.15 **Pathology readouts of complex in vitro models in safety assessments**

Nadine Stokar
F. Hoffmann-La Roche Ldt, Basel, Switzerland

New Approach Methodologies (NAMs) might provide more predictive insights into human organ function and disease pathophysiology, as well as more accurately predict safety and efficacy in drug development in future. One of those NAMs are Complex in vitro models (CIVM) that can address the need for more predictive human-based model systems in translational research. From a regulatory point of view, *in vitro* only safety assessments are currently performed and accepted for biotechnology-derived pharmaceuticals in the absence of a relevant animal species. However, the successful implementation and acceptance of CIVM requires their systematic characterization and validation to demonstrate to end-users as well as health authorities their fit for purpose or context of use (CoU). Examples of such *in vitro* safety packages have been successfully applied for bispecific T cell-recruiting antibodies (TCBs) or T cell receptor (TCR)-based immunotherapies acceptance into the clinic and to the market (i.e. Kimmtrak).

To be able to apply those CIVM in safety assessments in case a relevant animal species is available, the preclinical safety team needs to gain confidence with novel CIVM and their readouts. Pathologists have a significant and unique role in defining *in vivo* biology at the tissue, organ, and system levels, including dynamic physiology, responses to injury, and appropriate measures for extrapolation to *in vivo* responses. Therefore, pathologists can play a crucial role in characterization of CIVM towards their human tissue of origin, as well as validation towards the *in vivo* toxicological studies across animal species, also including humanized mice models. Histopathology is one of the main toxicological endpoints supporting entry into human (EiH) milestones in drug discovery. Similar to this approach, examples of histopathology and further incorporation of molecular tissue technologies into CIVM engineering and screening experiments i.e. (multiplex) immunohistochemistry, in situ hybridization, electron microscopy, mass spectrometry imaging) and automated / AI supported image analysis will be presented. Those molecular pathology technologies provide high throughput readouts for future *in vitro* efficacy and safety evaluations. Pathologists should be involved in model development, characterization and validation to ensure data harmonization and guide interdisciplinary teams to select the most relevant and translational models for future benefit-risk assessments in drug development.

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CASE PRESENTATIONS

CASE PRESENTATIONS

Morphology and Potential Mechanisms of Spinal Cord Findings Associated with ASO Administration in NHPs

Jairo Nunes[1], Valerie Dubost[2], Kuno Wuersch[2], Kelley Penraat[1], Diethilde Theil[3] and Kamal Kumar Balavenkatraman[2]

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The safety of a 2'-methoxyethyl (2' MOE) antisense oligonucleotide (ASO) was investigated in adult male and female cynomolgus monkeys (NHP) in a chronic toxicity study. The focus of this case report is to describe spinal cord (SC) findings in routine sections stained by hematoxylin and eosin (HE) and characterize the nature of the findings by immunohistochemistry (IHC), special histochemical stains (SHS), in-situ hybridization (ISH) and autofluorescence (AF). Monkeys were given lumbar intrathecal (IT) doses of the ASO in a chronic toxicity study and recovery animals were included. All animals survived to their scheduled necropsy. Transient dose-dependent clinical observations of absent spinal reflexes were noted at all doses, which fully reversed within 24 to 48 hours after dosing. Clinical pathology findings were limited to minimal increases in total protein and microalbumin levels at mid and high dose and minimally increases white blood cells (WBC) and presence of cytoplasmic magenta material in lympho-monocytic cells in the cerebrospinal fluid (CSF) at all doses. With the exception of the magenta material in lympho-monocytic cells within the CSF clinical pathology changes were not present in the recovery phase. Gross findings were limited to minimal sporadic enlargements of mandibular and mesenteric lymph nodes at the end of the dosing phase, which correlated with the presence of granular macrophages in the sinusoids, but no gross changes were noted in the SC.

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CASE PRESENTATIONS

Assessing the dimension behind the HE: novel technologies to complement tissue-based readouts for gene therapy

Bettina Amberg[1,2,], Sabrina Kehm[1,*], Alberto Valdeolivas[3], Petra Schwalie[3], Nadine Kumpesa[4], Fabian Koechl[4], Jose A. Galvan[1], Marion Richardson[1], Megana Prasad[4], Filip Bochner[5], Michael Otteneder[5], Matthias Selhausen[5], Petra Staeuble[1], Sven Rottenberg[2], Bjoern Jacobsen[1] and Kerstin Hahn[1]*

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Classical toxicology and discovery pathology has been dominated by assessments of HE slides, in situ hybridization, and immunohistochemistry. However, novel technologies such as omics-based readouts can provide valuable information and support mechanistic pathology assessments.
By using rAAV-based gene therapy as an example, in this case presentation we will show the dimension behind the HE by using multiplex immunohistochemistry, in situ hybridization, single nuclei sequencing and spatial transcriptomics.

CASE PRESENTATIONS

Direct Brain Delivery - Thinking Outside the Box

Deepa B. Rao and Kenneth A. Schafer
Greenfield Pathology Services, Inc

Signalment
- Species: Non-human Primate
- Sex: Female
- Study Type: Long-term Safety Tox Study
- Test Article: neuro-indication, biologic
- History: Post intra-parenchymal (brain) dosing, clinical signs included seizures. Not responsive to dexamethasone. Euthanized 4-hours post-dosing.
- Tissues Submitted: Brain, Heart, Lung

Traditionally, the blood-brain barrier has posed a hurdle for drug delivery, limiting the passage to allow only small selective molecules. With the recent advances in emerging neurotherapeutics, the delivery of large molecules to the neuroanatomical site remains limited to direct delivery such as intraparenchymal, intracerebroventricular, and intracisternal routes of administration. The following case presentation is of a single female cynomolgus monkey in a long-term safety toxicology study. The test article was a biologic for a neuroindication and the route of administration was intraparenchymal. Post-dosing, the animal was observed to exhibit clinical signs of seizure-like activity that was not responsive to dexamethasone and the animal was soon euthanized. Tissues available to the evaluating pathologist were limited to brain, heart, and lung.

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CASE PRESENTATIONS

Healthy appearing cynomolgus monkeys presented gross lesions suggesting a generalized granulomatous disease. All previously performed testing (PCR, Mantoux) revealed negative results. What went wrong?

Klaus Weber and Kristel Kegler
Anapath Services GmbH, Switzerland

Cynomolgus monkeys from a colony in South Vietnam were distributed to several Contract Research Organizations (CROs) by a European quarantine center. During necropsy, nodular lesions consistent with granulomatous inflammation were observed in the lungs and their associated lymph nodes in several animals. Additionally, some animals exhibited small grey-white foci in the liver, spleen, and less frequently in peripheral lymph nodes, kidneys, or myocardium, which were indicative of granulomas or abscesses. Several potential differential diagnoses were considered, including pseudotuberculosis (Yersinia and Corynebacterium), tularemia, or tuberculosis. The final diagnoses were established through necropsy, histology, and, in a subset of the animals, by employing PCR, ELISA, deep sequencing analysis, in conjunction with ultrasound examinations.
The presentation will cover clinical signs, gross and histopathology findings, as well as generated diagnostic data. An overview of the management of possible health issues among involved personnel, handling of samples, and affected regulatory studies will also be provided.

POSTER PRESENTATIONS

POSTER PRESENTATIONS

POSTER PRESENTATIONS

POSTER PRESENTATIONS

P01 | Chronic toxicity and carcinogenicity of AGIQ in Sprague Dawley rats by dietary exposure

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Introduction
alpha-Glycosyl isoquercitrin (AGIQ) is a flavonoid with antioxidative and tumor-suppressive capabilities that is marketed as a food additive in Japan. This study assessed the chronic toxicity and carcinogenicity of AGIQ in male and female SD rats following up to 5.0% dietary exposure.

Methods
Exposure for one (chronic toxicity study) or two (carcinogenicity studies) years.

Results
No AGIQ-related toxicity was observed except for yellow discoloration of bone. In the carcinogenicity study, a statistically significant increase in malignant gliomas of the brain or spinal cord was observed in female rats exposed to 5.0% AGIQ compared to controls (0/50 and 5/49 [10.2%], respectively). Based on cytological features and immunohistochemical positivity for the microglial marker Iba1 (ionized calcium-binding adaptor molecule 1), but not for astrocytic (GFAP, glial fibrillary acidic protein) or oligodendrocyte (Olig2, oligodendrocyte transcription factor 2) markers, neoplasms were categorized as “microglial tumor, malignant”. Recent historical control data (HCD) showed a total glioma incidence in female SD rats of 4/550 (0.7%, range 0-3.3%).

Conclusion
A Scientific Advisory Panel concluded that the Iba1-positive glioma could not definitively be attributed to AGIQ exposure, and thus has limited implications in predicting human cancer risk. The biological significance of these apparently rat-specific malignant tumors remains questionable since they are so rare in humans that the current classification system for human neural tumors does not include a “microglial tumor” category.

Impact statement
HCD indicate that gliomas occur spontaneously in rats, thus supporting the interpretation that gliomas observed in the current study are unrelated to treatment.

POSTER PRESENTATIONS

P02 | Mineral Fiber-Reinforced Plate Implantation in a Sheep Model: Long Term Safety and Bio-Integration In-Vivo Study

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[4] Meir Medical Center, Kfar Saba, Israel

Introduction

Traditionally, orthopedic implants were made of metal to provide mechanical strength and durability, essential for stable bone fixation. However, complications including implant migration, stress concentrations and bone-tissue loss, resulting in the need for hardware removal, drove the use of non-permanent polymer-based implants, which carry other risks, of adverse inflammatory reactions to degradation by-products. In this study, we evaluated the biocompatibility and bio-integration profile of a new class of mineral-fiber reinforced plates in a sheep tibia model.

Methods

Plates, made of continuous reinforcing mineral fibers bound together by PLDLA (50%w/w), were implanted bilaterally over the medial surface of sheep tibiae. Left tibiae underwent periosteal elevation, and right tibiae had intact periosteum. MicroCT and histopathology were performed at 13, 26, 52, 78, 104, 134-weeks(W) post-implantation. Overall cellular response, rate of bioabsorption (i.e., phagocytosis, M2-like macrophages/giant cell (MNGCs) infiltration), and mesenchymal ingrowth were graded according to ISO-10993-6(annex E).

Results

Mesenchymal ingrowth into the device wall was similar for both groups, and the cellular response consisted of anti-inflammatory M2-like macrophages and MNGCs. Adverse inflammation was not observed. Phagocytic activity started at 13W, peaked at 52W-78W, and resolved by 104W. Fibers were still evident at 78W and fully remodeled by 104W. By 134W, implants were fully bio-integrated.

Conclusion

This study demonstrates the safe use of fiber-reinforced implants in a bone plate application, with complete bio-integration with surrounding tissue and no toxicological adverse events.

Impact Statement

This new bone-fixation technology offers a solution to metal hardware complications and removals, while avoiding adverse inflammatory reactions reported for existing non-permanent implants.

POSTER PRESENTATIONS

P03 | Failure to gulp surface air induces swim bladder adenomas in Japanese medaka (Oryzias latipes)

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Introduction

Spontaneous swim bladder tumors are extremely rare in fish. However, Swim bladder carcinogenesis might be related to swim bladder inflation failure and/or spinal deformities.

Methods

In order to elucidate the effects of swim bladder inflation failure on swim bladder carcinogenesis, we investigated the sequential histopathological changes of swim bladders at 13, 24, 35, and 53 days post-hatch (dph) in medakas with an uninflated swim bladder, which was experimentally induced by denying access to the air-water interface between 0 and 6 dph. The reactive oxygen species (ROS) levels were measured at 24 dph. An uninflated swim bladder was induced in 47.3% of the fish denied access to the air-water interface (the denied group).

Results

The total incidence of swim bladder adenoma was 54.1% in the denied group; however, these tumors were observed in all fish with an uninflated swim bladder. In fact, these tumors were observed from 13 dph and onwards. The TBARS levels of the juveniles showed a 2.6-fold increase in fish with an uninflated swim bladder in the denied group compared to that in the control group.

Conclusion

It is speculated that swim bladder inflation failure has some effects on the gas gland to produce ROS, leading to DNA damage in the gas glandular epithelium, which develops into swim bladder adenomas. Consequently, it is concluded that denying access to the air-water interface between 0 and 6 dph in medaka is an easy method of inducing swim bladder tumors in a short-term period, and is a useful method for producing tumor-bearing fish.

POSTER PRESENTATIONS

P04 | Flubendazole exposure disrupts neural development and function of zebrafish embryos (Danio rerio)

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Introduction

Flubendazole (FBZ) is a benzimidazole anthelmintic widely used to treat parasitic infections by inhibiting the formation and function of microtubules by binding to tubulin. As its use has recently increased as not only anthelmintic but also anticancer drugs, the exposure of benzimidazole drugs to the environment has also increased. The negative impact of FBZ was observed to *D. magna*; however, effects of FBZ on aquatic organisms, especially toxic effects on aquatic vertebrate's neural development remain unclear.

Methods

This study aimed to investigate the potential developmental toxicity of FBZ during neural development using zebrafish model. Various assessments, including analysis of overall developmental changes, morphological abnormalities, apoptosis, gene expression alterations, axon length measurements, and electrophysiological neural function, were performed.

Results

FBZ exposure resulted in concentration-dependent effects on survival rate, hatching rate, heartbeat, and the occurrence of developmental abnormalities. Notably, FBZ-induced changes included reductions in body length, head size, and eye size, as well as the detection of apoptotic cells in the central nervous system. Gene expression analysis revealed upregulation of apoptosis-related genes (p53, casp3, and casp8), downregulation of neural differentiation-related genes (shha, nrd, ngn1, and elavl3), and alterations in neural maturation and axon growth-related genes (gap43, mbp, and syn2a). Additionally, shortened motor neuron axon length and impaired electrophysiological neural function were observed.

Conclusion

These findings provide novel insights into the potential risks of FBZ on the neural development of zebrafish embryos, emphasizing the need for risk prevention strategies and therapeutic approaches to address the environmental toxicity of benzimidazole anthelmintics.

POSTER PRESENTATIONS

P05 | Organ Weights and Spontaneous Microscopic Observations in the Testis from Sexually Mature Cynomolgus Macaques

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Introduction

When non-human primates represent the only relevant species for nonclinical safety evaluation of human medicinal products, potentially adverse effects on male fertility are typically assessed in repeat dose toxicity studies with sexually mature monkeys. As per regulatory guidance [ICH S6(R1)], default parameters for such an indirect assessment of fertility are reproductive organ weight and histopathology.

Methods

Data was collected from 69 vehicle-treated animals of 13 nonclinical safety studies that were conducted between 2016 and 2023. All animals were proven to be sexually mature by the presence of sperm in a semen sample. Their body weight was between 4.45 and 11.12 kg. 11 animals were of Mauritius origin, and 58 animals were of Asia mainland origin. Where appropriate, original diagnoses, that were reported in the study report, were translated into the current INHAND terminology.

Results

Approx. 1/3 of sexually mature male Cynomolgus macaques show a microscopic observation in the testis. The most frequently found observation is Degeneration/atrophy, tubule which occurs at an incidence of 32% but in most cases is minimal or slight. Only 1 out of 69 animals (1.5%) showed a bilateral Degeneration/atrophy of marked severity. Testis weight in sexually mature Cynomolgus macaques shows high variability with a range of 26.7g to 88.6g (based on the 2.5 and 97.5 percentiles) and does not correlate well with body weight.

Conclusion

We did not observe any relevant difference between Asia origin and Mauritius origin animals (with the caveat that the number of Mauritius origin animals in our investigation was low).

POSTER PRESENTATIONS

P06 | Opportunities for reducing recovery animals in NHP general toxicity studies

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Introduction

Non human primate (NHP) is a commonly used non-rodent species for safety assessment. There is an increasing awareness to reduce, refine and replace (3R) animal use. Therefore there is interest in finding opportunities to reduce the usage of NHPs. Recovery groups are used to assess the reversibility of toxicity findings, especially histopathology findings. However, recovery group use to assess reversibility is not always essential.

Methods

To streamline recovery group utilization, we conducted a retrospective analysis of NHP studies conducted by Roche for the past two decades.

Results

Our analysis showed that though recovery groups are routinely used in drug development programs, there are several opportunities to reduce their use by applying scientific judgment. We found that test article related histopathology findings, when present, often fully reversed or showed a trend towards recovery. Histopathology findings could often be predicted based on mechanism of action or could be attributed to modality related class effects, hypersensitivity reactions, adaptive effects, or stress related findings. Off-target effects were mainly seen with small molecules. Moreover the development of 'new' or 'unexpected' pathology findings during the progression of a project (short vs long term studies) was rarely observed.

Conclusion

In conclusion, though recovery group exclusion may not always be possible, scientific assessment by integrating information from the modality, indication, target biology, and prior toxicology and pathology data can help to promote judicious use of recovery animals and reduce overall animal usage.

POSTER PRESENTATIONS

P07 | The African Grass Rat Model of Metabolic Syndrome: Histomorphologic and Biomarker Study with Establishment of Serum Chemistry Reference Ranges

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Introduction

African grass rats are diurnal rodents commonly used in behavioral research. They spontaneously develop type II diabetes and metabolic syndrome (MS) on standard rodent chow. However, the clinicopathologic features and progression of this model are poorly characterized, and reference ranges do not exist. This study documents the pathophysiology, clinical pathology, and serum biomarker changes similar to human MS, and provides reference values for use in MS research.

Methods

Aged (11-15mo) and young (6-8wks) diabetic and young non-diabetic animals were used for clinicopathologic analysis. Following humane euthanasia, heart blood was obtained for serum chemistry and multiplex ELISA (insulin, leptin, ghrelin, adiponectin, HDL, LDL, cortisol, ACTH), and tissues collected for histopathology, special stains (VVG, ORO), and immunohistochemistry (insulin, glucagon, Casp3, Mac2).

Results

Hyperglycemia and elevated liver enzymes seen as early as 6wks correlated with macrovesicular hepatic steatosis. Aged animals were hyperglycemic, hypercholesterolemic, and hypertriglyceridemic. Multiplex ELISA showed elevated insulin, leptin, LDL, and decreased adiponectin and ghrelin. Hepatic steatosis and adrenocortical vacuolar degeneration was present in all animals, with atherosclerosis and islet alterations also observed in aged animals.

Conclusion

The African grass rat develops clinicopathologic lesions of metabolic disease as early as 6wks, with the spectrum of findings similar to human MS. We show dysregulation of biomarkers similar to human MS, and novel adrenal findings suggesting HPA axis disruption. Finally, we provide serum chemistry and biomarker reference ranges for this species important for MS research. This animal model provides a novel tool to understand progression of this disease, with advantages over traditional genetic, chemical and diet-induced models.

POSTER PRESENTATIONS

P08 | Comparison of hepatocyte proliferation in different models of liver injury

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is among the most common chronic liver diseases worldwide. Besides dietary models, chemical and surgical models mimic important disease hallmarks (i.e. steatosis, inflammation, fibrosis) for pharmaceutical research. Even though liver regeneration is known to occur in the cirrhotic stage and is involved in neoplastic transformation, regeneration enhancing liver function has not been systematically characterized in these models, especially in acute disease stages.

Methods

Three NAFLD mouse models were analyzed at different timepoints after injury (24-48h for APAP; 24- 96h for CCl4; 12-192h for hepatectomy). Using an AI system, immunohistochemical expression of proliferation markers Ki67, PCNA and BrdU was quantified specifically in hepatocytes and lesioned liver area was analyzed quantitatively based on Masson-trichrome stain.

Results

In CCl4, the highest numbers of Ki67- and BrdU- positive hepatocytes occur at 72h post injury; a significant increase in Ki67- positive cells can be detected as early as 48h in CCl4 but not APAP. PCNA-labelling partially reflects these findings but shows overall higher positive detections, also in controls. In partial hepatectomy, proliferation starts to increase at 36h and peaks at 42h; hepatocellular degeneration and necrosis is most widespread at 72h.

Conclusion

Hepatocyte proliferation is most pronounced at 48-72h after CCl4 and at 36-72h after 2/3 hepatectomy. BrdU and Ki67 reveal similar specific labelling, whereas PCNA is less specific for proliferation. Widespread liver lesions in hepatectomy are described to negatively impact regeneration. These data help to select among the mechanistically different injury models, time points and methods to assess hepatocyte proliferation.

POSTER PRESENTATIONS

P09 | Adenosquamous carcinoma with sebaceous gland differentiation in the Rat Mammary Gland

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Introduction

The morphology and hormone receptor pattern of a rare type of adenocarcinoma of the rat was investigated. The two female rats were control animals from a 52 week and a 104 week carcinogenicity study respectively.

Methods

Experimental design: Control animals from carcinogenicity study with Sprague-Dawley [Crl:CD(SD)] and HanBrl:WIST (SPF) rats were investigated to generate historical control data for these strains.
Materials and Methods: Tumor tissue of a female control rat from a 104 week carcinogenicity study which died spontaneously after 728 days on study was investigated. Macroscopically, a 15 mm diameter cyst was noted in the right inguinal side of the mammary gland. In another 52 weeks carcinogenicity study a female control rat was sacrificed on Day 372 (terminal kill) on study and a dark mass was observed in the left ventrocaudal side (19 mm in diameter). Hematoxylin-Eosin stained slides and serial sections stained immunohistochemically with antibodies against estrogen receptor, progesterone receptor and androgen receptor were evaluated.

Results

A malignant mammary gland tumor with features of sebaceous gland differentiation is described with the expression pattern of nuclear hormone receptors in tumor cells.

Conclusion

A new diagnosis “Mammary Carcinoma, adenosquamous with sebaceous differentiation” in the rat is proposed to be included in the INHAND nomenclature.

POSTER PRESENTATIONS

P10 | Assessment of different Methods to characterize the cell type specific AAV-PHP.eB vector distribution in the mouse brain

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Introduction

For central nervous system (CNS)-directed gene therapy, the assessment of the cell type specific vector distribution comprises a key readout. In our study, we determined the transduction or transgene protein expression in mouse brains transduced with AAV-PHP.eB vectors in major CNS cell types. We applied different methods to delineate advantages and disadvantages.

Methods

The data reported were generated from two AAV-PhP.eB intravenous injection studies using 5x1011 or 2x1013vg/kg. The expression cassettes contained CMV or CAG promoters and EGFP or its derivatives. We applied single nucleus RNA sequencing (snRNA-seq, 10X Genomics), RNAscope™ vector-specific in situ hybridization (ISH) combined with immunohistochemistry (IHC) using NeuN, GFAP, Iba1, Olig2, and CD34 specific antibodies or multiplex immunohistochemistry. All stainings were performed using the Leica BOND RX (Leica Biosystems) staining system and HALO software (Indica Labs) was used for data analysis.

Results

All methods identified AAV-PhP.eB transduction and/or transgene protein expression mainly in neurons and endothelial cells and fewer astrocytes and oligodendrocytes with higher sensitivity of ISH/IHC compared to snRNA-seq.

Conclusion

Our results underline that duplex ISH/IHC as well as multiplex IHC approaches represent an invaluable pathology readout to assess CNS cell type specific AAV vector distribution.

POSTER PRESENTATIONS

P11 | Neoplasia in laboratory-kept cynomolgus monkeys (Macaca fascicularis): Overall rare and diverse

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Introduction

In recent years, the development of new drug modalities has underlined the importance of nonhuman primates in research. Whereas information on neoplasia in laboratory-kept rodents is readily available, descriptions of unusual tumors in nonhuman primates are considerably less common. Herewith, the authors describe four cases of unusual neoplasms in cynomolgus monkeys of Asian and Mauritian origin kept in a research facility.

Methods

Four stock animals with different tumors were euthanized from 2018-2022 for clinical observations and animal welfare reasons. Full necropsies were conducted, tissue specimens were processed to block, sectioned, and stained with H&E. Immunohistochemistry was applied on selected specimens.

Results

Case 1 was a male with ataxia, lameness of the right leg, and paresis of the right arm. Pathology revealed a multilobulated mass in the cervical spinal nerve consistent with a schwannoma.
Case 2 was a juvenile female found dead. The mediastinum was expanded by an infiltrative mass associated with a cardiac tamponade. The mass was considered consistent with a thyroid carcinoma.
Case 3 was a female with reddening and swelling of the right eye. An invasive mass was noted in the globe at necropsy and was microscopically consistent with a retinoblastoma.
Case 4 was a male with body weight loss. The omentum was expanded by a malignant mesenchymal neoplasm. Histological features were indicative of a lipomatous tumor. No metastases were noted in these cases.

Conclusion

Neoplasms in cynomolgus monkeys vary widely with respect to tumor type, site of tumor development, and clinical symptoms and occur at any age and in both sexes. A diagnosis can be challenging and immunohistochemistry and human literature review are useful, especially if the tumor type remains questionable at first glance.

POSTER PRESENTATIONS

P12 | Antisense oligonucleotide BMN 351 restores motor function in a hDMDdel52/mdx mouse model of exon 51 skip-amenable Duchenne muscular dystrophy

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Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive degenerative muscle disease caused by frame-shift mutations in the DMD gene encoding dystrophin. A novel antisense oligonucleotide (BMN 351) was designed to selectively induce skipping in a DMD exon neighboring the frame-shift mutation, allowing the synthesis of a shortened and partially functional dystrophin protein.

Methods

BMN 351 was given to hDMDdel52/*mdx* mice by intravenous injection once weekly for 25 weeks at the doses of 6 or 18 mg/kg. Motor function and clinical/anatomic pathology data were evaluated at weeks 29 and 37 (i.e., 4- or 12-weeks post-dosing, respectively). Vehicle-treated hDMDdel52/*mdx* mice and healthy C57BL/6J wild type (WT) mice were used for comparison.

Results

Results were indicative of pharmacological efficacy of BMN 351 at each time point. Functionally, BMN 351 treatment at both doses improved overall gait scores, when compared with vehicle-treated hDMDdel52/*mdx* mice. Clinical pathology parameters such as AST, LDH and CK in BMN 351-treated mice at either dose were lower than in vehicle-treated hDMDdel52/*mdx* mice. Microscopic findings such as myofiber atrophy, necrosis, regeneration, mineralization, and inflammation showed a trend towards a dose-related lower incidence and severity in BMN 351-treated mice compared with vehicle-treated hDMD del52/*mdx* mice.

Vehicle-treated hDMDdel52/*mdx* mice had poor motor performance, high serum concentrations of AST, LDH, CK and microscopic muscle changes, consistent with the DMD phenotype. These changes were not observed in WT mice.

Conclusion

The functional and anatomic/clinical pathology improvement observed in hDMDdel52/*mdx* mice treated with BMN 351 are promising and support further development for clinical trials.

POSTER PRESENTATIONS

P13 | Analysis of blood parameters, bone marrow density and apoptosis in irradiated mice

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Introduction

Despite established knowledge that high-dose radiation can cause various adverse effects in the body, there is insufficient experimental evidence to confirm that low-dose radiation is entirely non-toxic. The aim of this study was to determine the effects of whole-body irradiation (WBI) on blood parameters, bone marrow density, and apoptosis in mice. To simplify the tedious and time-consuming manual calculation of cell counts and quantify cell density and apoptosis, we used machine learning-based image analysis by QuPath software.

Methods

Female C57BL/6 mice (n=120) were divided into four groups: group 1 served as the control, while groups 2, 3, and 4 received 0.5, 1, and 2 Gy, respectively, and were sacrificed at days 1, 3, and 7 after WBI. Blood analyses were performed, and the sternums and ileums were examined histopathologically.

Results

Although the body weight of the irradiated animals did not change, several blood parameters showed significant alterations, including decreased WBC and RBC counts and increased PLT counts. Histopathological examination revealed cellular damage in the sternum and ileum in groups 2, 3, and 4. Our machine learning-based image analysis showed that cell density was decreased and apoptosis in the sternum was increased, while in the ileum, apoptosis was increased.

Conclusion

Overall, WBI induced changes in blood parameters, cell density, and apoptosis in the sternum and ileum of mice, even at low doses. The use of machine learning-based image analysis makes it possible to quantify cell density and apoptosis accurately and rapidly, and it holds promise for the analysis of these changes after irradiation.

POSTER PRESENTATIONS

P14 | Recommendations for GLP-Conform Archiving of Whole Slide Images

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Introduction

In recent years, working groups of toxicologic pathology societies (ESTP, STP), consortium-driven direct interactions with regulatory authorities and efforts made by individual institutions have helped to develop a general understanding of elements required for the GLP-conform use of digital pathology.
To date only a few institutions have claimed to use digital pathology under GLP, and only a minority of these have been inspected by national GLP authorities, generating little feedback to build upon.

Methods

An informal pre-competitive inter-laboratory exchange forum was set up in fall 2022 by interested toxicologic pathologists from the pharmaceutical and agrochemical industry and CROs, with the aim to share and convert the collective knowledge of theoretical requirements into aligned practical recommendations and solutions for the most critical aspects of digital pathology workstream GLP validation.

Results

Archiving of whole slide images (WSI) must be considered in the context of raw data generation. If archival of WSI is required (primary evaluation, retrospective peer review), in addition to the archival of glass slides, the processes to retain original electronic data generated by the computerized system as well as procedures to assure data integrity need to be defined according to the respective OECD documents.

Conclusion

The group recommends using DICOM format for back compatibility reasons. Additionally, processes such as archival log, duration of archiving as well as recovery and disaster recovery need to be defined. Finally, the decision of using either cloud solution or physical address of the data center for archiving purposes needs to be based on a suitable risk assessment.

POSTER PRESENTATIONS

P15 | Recommendations for the Utility of Equivalency and Concordance Studies in Digital Pathology

Jonathan Carter[1], Ute Bach[2], Julie Boisclair[3], Thomas Forest[4], Peter Hall[5], Charles Halsey[6], Charlotte Lempp[7], Pierre Maliver[8], Patricia Neves[9], Moritz Radbruch[2], Frédéric Schorsch[9], Charlotte Dalsgaard[10], Xavier Palazzi[6], Gabriele Pohlmeyer-Esch[7]

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Introduction

To date only a few institutions have claimed to use digital pathology under GLP, and only a minority of these have been inspected by national GLP authorities, generating little feedback to build upon.

Methods

An informal pre-competitive inter-laboratory exchange forum was set up in fall 2022 by interested toxicologic pathologists from the pharmaceutical and agrochemical industry and CROs, with the aim to share and convert the collective knowledge of theoretical requirements into aligned practical recommendations and solutions for the most critical aspects of digital pathology workstream GLP validation in common use cases.

Results

With regard to the utilization of comparative examinations of glass slide versus whole slide image, it was this group's opinion that a blinded concordance study (examination of glass slides and WSI, with an intervening wash-out period, and statistical analysis for concordance of the data generated from each media type) was primarily applicable as a proof of concept and not a prerequisite for GLP-compliant digital peer review or primary read. However, a need for published documentation of concordance studies from the toxicologic pathology industry to further support GLP digital primary read was identified. The requirement for documented pathologist approval that WSI are fit for purpose (GLP digital peer review and primary read) was, therefore, considered to be met by an equivalency study (non-blinded comparison of glass slides and WSI to qualitatively confirm equivalence based on assessment of critical features and/or lesions).

Conclusion

N.A.

POSTER PRESENTATIONS

P16 | Recommendations for the Qualification of Instruments in Digital Pathology

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Introduction

In recent years, working groups of toxicologic pathology societies (ESTP, STP), consortium-driven direct interactions with regulatory authorities and efforts made by individual institutions have helped to develop a general understanding of elements required for the GLP-conform use of digital pathology for peer reviews and primary reads. To date only a few institutions have claimed to use digital pathology under GLP, and only a minority of these have been inspected by national GLP authorities, generating little feedback to build upon.

Methods

An informal pre-competitive inter-laboratory exchange forum was set up in fall 2022 by interested toxicologic pathologists from the pharmaceutical and agrochemical industry and CROs, with the aim to share and convert the collective knowledge of theoretical requirements into aligned practical recommendations and solutions for the most critical aspects of digital pathology workstream GLP validation in common use cases.

Results

The group recommends that instrument qualification of scanners and pathologist workstations be based on a set of predetermined specifications by both the vendor (installation and operational qualification) and end-user requirement specifications (user acceptance testing).

Conclusion

Spatial and color calibration of scanners (calibrating displayed lengths against known values) are discussed. The group felt that the monitor and the viewing environment are a point of concern. This can be addressed via a QC slide, “point of use QA” approaches, color calibration, and/or via equivalency studies. Options and open questions are discussed.

POSTER PRESENTATIONS

P17 | Pre-Requisites to the Conduct of a Non GLP Digital Peer Review on a GLP study

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Introduction

In recent years, working groups of toxicologic pathology societies, consortium-driven direct interactions with regulatory authorities, and efforts made by individual institutions have helped to develop a general understanding of the elements required for the GLP-conform use of digital pathology.
To date only a few institutions have claimed to use digital pathology under GLP, and only a minority of these have been inspected by national GLP authorities, generating little feedback to build upon.

Methods

An informal pre-competitive inter-laboratory exchange forum was set up in fall 2022 by interested toxicologic pathologists from the pharmaceutical and agrochemical industry and CROs, with the aim to share and convert the collective knowledge of theoretical requirements into aligned practical recommendations and solutions for the most critical aspects of digital pathology workstream GLP validation.

Results

One scenario discussed was not claiming GLP compliance for digital peer review on a GLP study. An in-depth assessment of the existing published regulatory guidance and their requirements was performed. The analysis suggested that not claiming GLP compliance for a digital peer review should be acceptable as long as the overall integrity of the GLP study is maintained. It was considered this scenario represented a low regulatory risk.

Conclusion

The poster summarizes the conclusion of the regulatory review, possible digital workflows with their respective stakeholders, and lists the mandatory and optional, site- and study-specific requirements that need to be in place before, during and after the conduct of the digital peer review. Examples of wording for SOPs, study plans, and study reports are suggested.

POSTER PRESENTATIONS

P18 | MEN-like Syndrome: Does it occur in rats? - An Analysis of Data from the RITA Database

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Introduction

Multiple endocrine neoplasia (MEN) is a condition which encompasses several distinct syndromes featuring at least two tumors of endocrine glands, mainly of neuroendocrine origin. While proliferations of neuroendocrine origin in humans are related to a genetic background (MEN Syndromes), the background in domestic and laboratory animals in most cases is unknown (MEN-like Syndromes). An analysis of RITA data was performed to investigate whether the condition of a MEN-like syndrome may occur also in Wistar- and Sprague Dawley (SD) rats.

Methods

Substrains of control Wistar and SD rats were analyzed for proliferative neuroendocrine lesions from adrenals, pancreas, thyroid, parathyroid and pituitary. The diagnoses followed the criteria as described in INHAND. Animals with single organ versus multiple organs affected were counted and compared to expected values. In case of significantly increased multiple affections the combinations of organs known from MEN Syndromes were evaluated in search of MEN-like Syndromes. Co-occurrence in two organs versus one organ was analyzed as compared to expected values.

Results

In male Wistar rats, significant results were observed in pancreas and thyroid. In male SD rats, similar results were seen for the thyroid, adrenal, and pancreas. An increased co-occurrence of adrenal and thyroidal lesions was clearly evident for both male Wistar and SD control rats.

Conclusion

Even though no clear evidence for the existence of MEN1-like syndromes was given, the results from males of both breeds showed a MEN2-like pattern. This analysis indicates the value of detailed metadata of the RITA database.

POSTER PRESENTATIONS

P19 | ESTP & STP Working Group "Complex In Vitro Models & Pathology" - Interdisciplinary Team Driving New Translational Platforms Across Industry

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Introduction

Complex in vitro models (CIVM), under the umbrella of New Approach Methodologies (NAMs) address the need for more predictive human-based model systems in translational research. The recent passage of the FDA Modernization Act 2.0 by the US congress elevates the potential for replacement of animals in toxicity testing with NAMs.

Methods

The European Society of Toxicologic Pathology (ESTP) and Society for Toxicologic Pathology (STP) are working together on the ESTP Pathology 2.0 Subgroup "Complex in vitro model (CIVM) & Pathology" to bring together different scientists and to increase the interdisciplinary communication between engineers, biologists and pathologists in industry on the topic of CIVM and their use in drug and plant protection product development.

Results

Preclinical pathologists in industry are trained in comparative animal biology, which provides them with the expertise to bridge between animal models and humans. This knowledge can be expanded to CIVM. Nevertheless, pathologists need education and exposure to CIVM by the model engineers and users, to increase confidence in morphologic evaluation and incorporation of tissue technologies into CIVM experiments. Here we aim to share cross industry examples from working group members that describe on how tissue technologies and analytics can support CIVM engineering and the characterization/validation of their context of use (CoU).

Conclusion

This will require fostering interdisciplinary collaborations, which are imperative to increase industry confidence in the use and application of CIVM for efficacy, mechanistic investigation, safety evaluation, and risk assessment in drug discovery and development and the agrochemical sector.

POSTER PRESENTATIONS

P20 | Histology-guided engineering and characterization of complex in vitro models of blood-brain barrier for efficacy/toxicity in early drug development

Luisa Bell, Martina Pigoni, Roberto Villasenor, Christelle Zundel, Petra Stäuble, Nadine Stokar
Roche, Basel, Switzerland

Introduction

Complex *in vitro* models (CIVM) of the blood-brain barrier (BBB) could facilitate effective testing of drug candidates targeting the brain early in the drug discovery process. Temporal and phenotypic histomorphological readouts of CIVMs offer an excellent opportunity to address discrete mechanistic questions at the cellular and/or tissue level and enable comparison to the standardized histomorphological readouts of *in vivo* studies.

Methods

Here, we present an array of tissue technologies (Haematoxylin Eosin staining, HE; immunohistochemistry, IHC; immunofluorescence, IF) that enabled characterization/validation and histology-guided engineering of CIVM of the BBB, that was previously developed for investigating receptor-mediated transcytosis (Simonneau et al., 2021).

Results

Expression of relevant cellular BBB markers was observed both in human brain tissue samples and BBB organoids by IHC. Multiplex IF provided spatial information during the engineering steps for a correct assembly of the three cell types to a functional BBB. We could conclude that our BBB organoids did not recapitulate the morphology and cell composition of the human BBB, demanding an improved cell source for brain endothelial cells. To troubleshoot, different protocols of endothelial cell generation were developed with BBB transwells. Histologic evaluation of a HE staining was used to monitor the assembly of a monolayer of brain endothelial cells in the transwell.

Conclusion

Standard morphologic readouts provide a quick and simple overview during engineering of novel CIVM enabling their characterization towards their tissue of origin and input cell sources. In turn, pathologists can bring their expertise to improve the translatability of preclinical research and relate outcomes to human patients.

POSTER PRESENTATIONS

P21 | Pathology 2.0: a collaborative working group to increase awareness of cutting-edge technologies in experimental and toxicologic pathology

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Introduction

The ESTP Pathology 2.0 working group fosters a collaborative network of pathologists and non-pathologists from different organizations across Europe and US. The main purpose is to share knowledge, skills, and experience among the society members about relevant topics of interest for the pathology community.

Methods

Knowledge sharing comes in the form of webinars, publications, surveys, and presentations at international congresses. The partnership and coordinated collaboration between individuals from different organizations also creates a space that embrace innovation and supports the development and implementation of new technologies.

Results

Pathology 2.0 is organized in 5 different subgroups which work synergistically in different goals and strategies. The subgroups are: 1) Molecular Pathology; 2) Multiplexing; 3) Spatial OMICS; 4) Mass Spectrometry Imaging; 5) Complex in vitro models & Pathology. Since first created, Pathology 2.0 has been continuously expanding and now counts more than 40 members. Most importantly, the working group is always open to new members and ideas to continue advancing knowledge, promoting collaboration and ultimately bringing a positive impact to the toxicological pathology field.

Conclusion

This poster gives an overview of the Pathology 2.0 activities and goals achieved during the last year.

POSTER PRESENTATIONS

P22 | Challenges and Opportunities in Use of the Minipig for Nonclinical Pharmaceutical Development: Results of the Second IQ DruSafe Minipig Survey

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Introduction

As part of IQ (International Consortium for Innovation and Quality in Pharmaceutical Development), a not-for-profit organization of pharmaceutical and biotechnology companies, the Preclinical Safety Leadership Group (DruSafe) decided to survey its members on their use of minipigs for nonclinical assessment in 2014. This survey indicated minipig use was largely limited to studies evaluating small molecule dermal products.

Methods

N/A

Results

An updated IQ survey, prepared and distributed in 2022, revealed an incremental increase in minipig use, primarily in the development of proteins, oligonucleotides, medical devices, and small molecules. Despite this increase, minipig still represents generally ≤5% of all nonrodents used in pharmaceutical development. Perceived key challenges to wider use of minipigs are: (i) higher test article requirement; (ii) lack of historical control data (HCD); (iii) lack of relevant reagents/assays to assess pharmacology or toxicity; and (iv) limited number of efficacy models. IQ companies also expressed concerns regarding in-house capabilities, training, and handling of minipigs as well as uncertainty regarding capabilities and experience at CROs. The EU- and UK-based member companies commented that there is an increased consideration of utilizing minipigs due to ethical considerations related to monkey and dog usage. Three CROs confirmed the trend of increase in minipig use. They experienced similar challenges as IQ companies, but opine HCD generation, CRO expertise, availability of minipigs, and reagents/assays have improved.

Conclusion

The 2022 IQ DruSafe survey results indicate that many of the concerns previously identified persist. Industry-wide efforts to address these challenges may promote consideration of minipigs as nonrodent species for nonclinical pharmaceutical development.

POSTER PRESENTATIONS

P23 | Spatial high-throughput single cell RNAseq method with feature barcoded microbeads and AI-guided image analysis

Markus Sainio[1], Päivi Saavalainen[1], Tuomas Väisänen[2], Hanna-Kaisa Sihvo[2], Laura Lehtinen[1], Katarzyna Leskinen[1], Heli Pessa[1], Kirsi Sepponen[1], Linnea Hartwall[1], Ville Jokinen[1]

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Introduction

Spatial single cell RNAseq is an emerging tool in biomedicine, but linking cell images or functional read-outs with RNAseq data is still challenging in high throughput arrays.

Methods

We developed an AI-assisted method to barcode any pico/nanowell arrays spatially with coloured microbeads bearing photocleavable oligos with feature barcode (FBC) and poly-A tail. A mix of 20 visibly coloured microbeads (4-10um) were loaded on arrays with over 100.000 wells of 15-50um diameter. Chips were imaged using a microscope scanner. We developed a three-layer AI model to detect wells and bead types within the wells. The scanned arrays were then loaded with cell lines or cryosectioned tissues. Cellular mRNA from lysed cells and UV-released feature sequences from FBC beads were both captured by separate cell barcode (CBC) beads. 3' end RNAseq libraries were sequenced with Illumina and the gene expression profiles for each CBC was linked with the FBC data and matched with the array map created with the AI model.

Results

Random loading of 20 FBC bead types in wells produces theoretically over 1 million possible unique combinations. Our AI model observed that with an average loading of 8 beads per well, over 50% of 50um wells had a unique FBC bead combination. We demonstrated he feasibility of the method with mixed cell lines and mouse brain cryosections.

Conclusion

Our new method enables spatial analysis of tissue cryosections and cell suspensions in high throughput arrays, allowing also functional readouts from the wells.

POSTER PRESENTATIONS

P24 | Automated histomorphologic artificial intelligence (AI) readout for toxicity evaluation in blood-brain barrier organoids

Luisa Bell, Elena Kassianidou, Roberto Villasenor, Nadine Stokar
Roche, Basel, Switzerland

Introduction

Human complex *in vitro* models (CIVM) address the need for more predictive toxicology models that might increase future clinical success in drug development. Digital tools enable high throughput and automation combined with evaluation of single cell morphology as it is the standard for histopathologic evaluation performed by pathologists today. As a proof-of-concept model, we used the blood-brain barrier (BBB) organoids, an excellent CIVM to screen for efficacy and toxicity in brain delivery of biologics.

Methods

We developed an artificial intelligence (AI) tool using Visiopharm software to automatically detect apoptotic/necrotic nuclei in BBB organoid 40x whole slide Haematoxylin and Eosin (HE) images. The ground truth included Caspase-3 immunohistochemistry (IHC) as well as evaluation of apoptotic/necrotic nuclei on HE slides (40 randomly chosen organoids) by three blinded ECVP board-certified veterinary anatomic pathologists. For validation, we treated BBB organoids with different concentrations of staurosporine to induce apoptosis and compared the algorithms' performance towards a standard low magnification (5x) caspase 3/7 assay.

Results

As a result, our AI algorithms detected the apoptosis as well as the 5x caspase assay, and additionally provided a percentage of apoptosis at the single cell level instead of an arbitrary fluorescence signal. It further added single cell and spatial resolution, digitalization, automation, high throughput and reproducible components.

Conclusion

Our proof-of-concept study showed that our label-free end-to-end workflow presents a novel tool to automatically screen for apoptosis/necrosis in BBB organoids at a single-cell level, and can be applied to future toxicity studies performed with BBB organoids.

POSTER PRESENTATIONS

P25 | Improved tolerability with the vascular access button (VAB) vs. vascular access harness (VAH) method in the rat

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Introduction

The vascular access harness (VAH) and vascular access button (VAB) are currently used in infusion studies in rats.

Methods

Thirty-six male Wistar rats were split into 4 groups and submitted to 13-week intermittent infusion of saline (0.9% NaCl) or mannitol (50 mg/mL) at 1 mL/kg/hour, 5 days a week. Each solution was administered either with VAH or VAB system. Clinical signs, body weight, food intake, infusion incidents, clinical pathology, gross and microscopic findings were evaluated.

Results

There was no mortality. Independent of the formulation infused (saline or mannitol), catheter occlusion occurred in 11/18 VAH-equipped rats and 3/18 males VAB-equipped rats. Catheter disconnection occurred in 10/18 VAH-equipped rats. Skin ulceration due to the harness was noted in 3/18 VAH-equipped rats.
At euthanasia, lower white blood cell counts were noted in VAB-equipped rats.
At necropsy, there were firm consistency of catheterized vena cava, enlargement of left iliac lymph nodes, cutaneous scab and spleen enlargement in VAH-equipped rats and not in VAB-equipped rats. Microscopically, findings were observed at the infusion site (thrombus and vascular/perivascular inflammation), iliac lymph nodes (increased cellularity of germinal centers and plasma cells), spleen (extramedullary hematopoiesis), kidneys (inflammation) and lungs (thrombus) at lower severity/incidence in VAB- than VAH-equipped rats. Bacterial colonies were observed at the infusion site in VAH-equipped rats only.

Conclusion

In-life and post-mortem findings were indicative of improved tolerability with the VAB perfusion system, which also had animal welfare advantages because it allowed animal group housing.

POSTER PRESENTATIONS

P26 | Macroscopic and Microscopic Evaluation of Injection Sites for Long-Acting Injectables- Proposed Guidelines on Terminology

Keith Nelson[1], Elodie Drevon-Gaillot[2], Anna-Lena Frisk[3], Vijaykumar Kale[4], Charlotte Keenan[5], Lars Mecklenburg[6], Marjolein Heerden[3]

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Introduction

Long-acting injectables (LAIs) are typically formulated in a liquid solution or suspension administered by subcutaneous (SC) or intramuscular (IM) injection that may form a depot from which the active agent is slowly released over time, or as solid or eluting implantables. Assessment of these injection sites should include both the acute and chronic response to the LAI and their recovery. Recording of LAI findings at the macroscopic level is generally descriptive, with size, color, and other characteristics noted.

Methods

Microscopic description may be split between the central changes at and around the depot such as presence of any delivery material, pseudocyst, and peripheral/associated changes such as inflammatory cell infiltrate, inflammation, fibrosis, granulation tissue, necrosis, hemorrhage, hyperplasia, or degeneration/regeneration. Diagnoses may also be organized by tissue subcompartments (eg. epidermis, dermis, and subcutis/muscle). We here outline basic terminology and propose several additional terms that are not currently in the Standard for Exchange of Nonclinical Data (SEND) and International Harmonization of Nomenclature and Diagnostic Criteria for Lesions (INHAND) for consideration. Readers are invited to review and evaluate the proposed additional terminology and vote for preferred terms, using either the provided physical materials or the QR code-accessible survey.

Results

N.A.

Conclusion

The results from this poster-based survey will be considered and discussed in the post-meeting 10th Annual ESTP International Experts workshop on Long Acting Injectables as we move forward with proposing consistent standard terminology in the evaluation of injection site histopathological findings.

POSTER PRESENTATIONS

P27 | SlideQC: an Automated Artificial Intelligence-Powered Artifact Detection Tool for Quality Control of Whole-Slide Digital Pathology Images

Daniela Rodrigues[1], Erik Burlingame[1], Corey Babcock[1], Vlado Ovtcharov[1], Stefan Reinhard[2], Therese Waldburger, Daniel Martin[3], Suzana Couto[3], Inti Zlobec , Peter Caie[1]

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Introduction

Toxicity screening of tissue samples using whole-slide imaging and quantitative image analysis allows identification of morphological changes consistent with cellular stress, damage, and necrosis. However, the presence of artifacts, often introduced during sample preparation and scanning, can negatively impact morphological assessment, and threaten the accuracy of image analysis-based assays. For automated quality control, we developed the SlideQC network to segment artifacts in Haematoxylin and Eosin (H&E) and immunohistochemistry (IHC) stained whole-slide images (WSI).

Methods

SlideQC was developed using 2499 annotations across 302 H&E and IHC stained WSI for artifacts such as air bubbles, dust/debris, folds, out-of-focus, and pen marker. SlideQC performance was evaluated on 432 annotations (tissue and artifact) from 73 external H&E (HistoQC Repo) and IHC (LYON19) external test cohort images.

Results

SlideQC showed high precision, recall, and F1-score with average values of 0.94, 0.90, and 0.91, respectively, over pixel-level annotations. The average recall per artifact type was 0.84 for air bubbles, 0.91 for debris/dust, 0.84 for folds, 0.98 for pen marker, and 0.97 for out-of-focus regions. Analysis of a 15x15mm area with SlideQC takes an average of 1 minute and 41 seconds.

Conclusion

Whole-slide imaging and quantitative image analysis play a key role in toxicology research, helping to dissect biological processes and understand mechanisms of toxicity. SlideQC supports the identification and exclusion of artifact regions, allowing to triage slides with a high percentage of artifacts or to exclude them from subsequent analysis. Using SlideQC can result in significant time savings in comparison with manual quality control.

POSTER PRESENTATIONS

P28 | Real-time messaging of pathological diagnoses from toxicology laboratory information management system (LIMS) to cloud-based digital pathology web application

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Introduction

Exchanges between toxicologic pathologists have always been critical to establish diagnoses, especially to reinforce the interpretation during peer reviews. Access to Whole Slide Images (WSI) is now easier and can be done by different pathologists from different places at a chosen time.
Here we present an innovative feature which allows pathologists to have access simultaneously to the WSI and their pathological diagnoses updated in near real-time from the internal laboratory information management system (LIMS).

Methods

Toxicology studies were managed on Pristima (Xybion) LIMS. WSI were generated on an Aperio AT2 scanner (Leica Biosystems), filenames were automatically created from the glass slides' barcodes to encoding relevant identifying metadata including study number, animal number and tissue abbreviations. WSI were uploaded to a Concentriq (Proscia), web application hosted on a Bayer-private Amazon web service (AWS) account.

Results

First, automated population of the respective WSI metadata fields (study number, animal number, and tissue abbreviations) in Concentriq took less than a minute after WSI upload. Then, Pristima messaging module identified each WSI in Concentriq specific to this combination of study number/animal number/tissue. Finally, Pristima messaging module sent the correct pathological diagnoses to the dedicated metadata field next to each corresponding WSI in less than 2 minutes.

Conclusion

The developed interface Pristima-Concentriq offers an innovative way to exchange on interpreted WSI from a web application easily accessible to users, from anywhere around the world. This interface relies on a defined WSI naming convention and is expected to improve study case evaluation and image analysis research in the future.

POSTER PRESENTATIONS

P29 | Pathohistological studies to exclude immunotoxic effects of the TherVacB vaccine of a murine AAV-HBV model in an immunotoxicity study

Anke Hoffmann[1], Sina Riemschneider[2], Kristin Klose[2], Olga Antoniadou[2], Volker Bruns[3], Markus Kreuz[2], Anna Kosinska[4], Frank Thiele[4], Marian Wiegand[4], Ulrike Protzer[4], Joerg Lehmann[2]

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Introduction

Currently, there is no therapeutic approach that is capable of curing chronic hepatitis B virus infection (HBV). Therefore, a novel heterologous protein prime/vector boost vaccination approach (TherVacB) has been developed which was studied for potential immunotoxicity using a transgenic AAV-HBV mouse model. Here, we demonstrate the effects of TherVacB administration on liver histopathology from this GLP study.

Methods

Liver tissue samples from 120 AAV-HBV-transduced C57BL/6JRj mice were semi-quantitatively assessed by haematoxylin/eosin staining for vaccine-dependent findings, e.g., inflammation, cell death, and fibrosis. To characterize the inflammation with regard to T-cell infiltration, CD3, CD4, and CD8a T cells were immunohistochemically stained and quantitatively evaluated using the new image analysis software MICAIA.

Results

No severe abnormalities of inflammation and cell death were found in control and treatment groups of both sexes. For these parameters, minimal to minor severity were observed in the majority of animals, and a part of the animals did no show any abnormalities for cell death. Also, fibrosis was not detected in any case. A significant increase in CD3 and CD8a T cells and a consistant increase in CD4 T cells were observed in the treatment compared to the control group.

Conclusion

The histopathological examination of mouse liver sections did not reveal any evidence of immunologically induced hepatotoxicity potentially caused through administration of the TherVacB vaccine. The massive infiltration of T cells, in particular CD8a T cells, detected by immunohistochemistry, indicates a strong cytotoxic immune response that supports viral elimination and represents an essential step towards functional cure of chronic HBV infection.

POSTER PRESENTATIONS

P30 | Tolerability Assessment of Injection Site Reactions for Long-Acting Injectables

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Introduction

Long-acting injectables (LAIs) are designed to provide a consistent delivery of the test article over a long period of time, reducing the dosage frequency and increasing patient dosing adherence and efficacy. LAIs can be administered by subcutaneous, intramuscular, or intra-articular routes depending on formulation and indication. Different delivery material formulation types such as suspensions, oil solutions, polymer embeddings in microparticles or implants can impact the duration and/or severity of local injection site reactions (ISRs). One of the goals of the LAI nonclinical safety studies is to evaluate the local tolerability of LAI formulations for the purpose of human risk assessment. In this poster session, we present several selected case studies to assess local tolerability of the test article administered as LAIs in various models and situations. Readers are invited to provide feedback on these examples of tolerability assessment using either the provided physical materials or the QR code-accessible survey. The feedback from this and another poster on terminologies used in evaluating and reporting the injeciton site histopathological findings will be discussed in the post-meeting workshop on long-acting injectables.

Methods

NA

Results

NA

Conclusion

The results from this poster-based survey will be considered and discussed in the post-meeting 10th annual ESTP international experts workshop on long-acting injectables as we move forward with proposing a consistent approach (points-to-consider) to local tolerability assessment in nonclinical studies.

POSTER PRESENTATIONS

P31 | Unexpected Acute Toxicity of a Monoclonal Antibody in Rats

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Introduction

The toxicological profile of a human monoclonal antibody was assessed in a 4-week toxicology study in rats.

Methods

Five male rats per group were administered weekly intravenous doses of 30, 200 and 600 mg/kg or weekly subcutaneous doses of 200 mg/kg. Study endpoints were tolerability, toxicokinetics, clinical pathology, and histopathology.

Results

On Day 1, severe clinical signs involving decreased activity, cold skin, and recumbent posture were observed in two rats at 200 mg/kg IV, requiring euthanasia. On Day 2, two rats at 600 mg/kg IV were found dead and the remaining animals in that group were euthanized due to severe clinical signs similar to Day 1. At the unscheduled necropsies, red discolorations of various organs, decreased red blood cell mass and platelet counts and increased total bilirubin associated with complement activation (C3a) were observed, indicative of an acute immune response and/or interaction with red blood cells leading to hemolysis. Histopathology findings involved degeneration and/or necrosis and/or hemorrhages in liver, hemorrhages and/or edema in lungs, and erosions and/or hemorrhages in stomach, suggesting an immunologic effect of the antibody. The remaining three rats at 200 mg/kg IV recovered and tolerated subsequent dosing until the end of the study without major pathology findings.
Rats administered 200 mg/kg SC exhibited inflammation at the SC injection site characterized by macrophages, neutrophils and fibroplasia, also suggesting an immunologic effect of the antibody. There were no other pathological changes.
Toxicokinetics at doses ≥ 200 mg/kg IV were characterized by increased antibody clearance which could be indicative for aggregation or immune complex formation. SC bioavailability at 200 mg/kg was 9%.

Conclusion

In vitro immunosafety assays could not explain the cause of toxicity. The rapid onset of toxicity, the histological and clinical pathology findings together with complement activation and increased antibody clearance suggest complement-dependent immune toxicity likely associated with immune complex formation.

POSTER PRESENTATIONS

P32 | Intracranial meningioangiomatosis in two adult dogs: histological and immunohistochemical study

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Introduction

Meningioangiomatosis is a rare proliferative disorder of the central nervous system with meningothelial/fibroblastic and vascular components that has been described in humans and animals. In dogs, lesions are found primarily in the leptomeninges of the brainstem but can also occur in the cervical and thoracolumbar spinal cord. Two cases of adult dogs that clinically showed neurological signs compatible with right vestibular syndrome and seizures are described. In both cases, the magnetic resonance identified intracranial lesions that caused mass effect, located at the level of the midbrain in the first case and in the cerebral and right cerebellar hemisphere in the other. The clinical presumptive diagnoses were of infiltrative neoplasm versus inflammation.

Methods

Both animals were euthanised, post mortem examination was performed with histophatological and inmunohisthochemical studies.

Results

Macroscopically a focal extensive area of discoloration and softening was observed in the midbrain of the first dog and in the right cerebelar and cerebral hemispheres of the second dog. Histologically, leptomeinges were disrupted by a plake-like neoplastic infiltration composed of meningiothelial cells that extend from the subarachnoideal space into the adjacent parenchyma along the microvasculature and with a variable vasoformative component. Neoplastic cells were spindle shaped with lack of atypia and arranged in angiocentric pattern of distribution. In the first case the neoplastic growth was highly invasive and caused considerable parenchymal degeneration with loss of abundant cellular components. Inmunohistochemical study for the detection of vimentin, CD31 and F8 were positive.

Conclusion

Histological and inmunohistochemical studies confirmed, in both cases, the final diagnosis of intracranial meningioangiomatosis.

POSTER PRESENTATIONS

P33 | Therapeutic Oligonucleotides: A Retrospective Evaluation of Non-Clinical Toxicology Data Obtained at Charles River Laboratories France

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Introduction

RNA-targeting oligonucleotides 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 anatomic pathology 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).
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 in laboratory animal species.

POSTER PRESENTATIONS

P34 | Guide for Combining Primary Tumors for Statistical Analysis in Carcinogenicity Studies

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Introduction

This guide was created at the request of the U. S. Food and Drug Administration (FDA) by a Working Group of biopharmaceutical experts from the Society of Toxicologic Pathology (STP), FDA, and members of the Standard for Exchange of Nonclinical Data (SEND) initiative, with a primary goal of assisting pharmacology/toxicology reviewers and biostatisticians in statistical analysis of nonclinical tumor data. The guide will also be useful to study and peer review pathologists in interpreting tumor data. This guide provides a user friendly, higher-level hierarchy of tumor types or categories correlating the tumor names from the International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) publications with those available in the NEOPLASM controlled terminology (CT) code list in SEND. The version of CT used in a study should be referenced in the nonclinical study data reviewer's guide (SDRG) (section 3.1) of electronic submissions to the FDA. The tumor combination guide instructions and examples are in a tabular format to make informed decisions for combining tumor data for statistical analysis. The guide is targeted for publication on the Clinical Data Interchange Standards Consortium (CDISC) website by the end of 2023. The strategy for combining tumor types for statistical analysis is based on scientific criteria gleaned from the current scientific literature; as SEND and INHAND terminology and information evolve, this guide will be updated.

Methods

NA

Results

NA

Conclusion

NA

POSTER PRESENTATIONS

P35 | AI-based testicular staging in dogs from nonclinical toxicity studies

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Introduction

Spermatogenic staging of tubules via histologic examination is typically used to evaluate testicular toxicity in non-clinical studies. However, this process is time-consuming, non-exhaustive, and shows inter-observer variability between pathologists. Artificial intelligence (AI) offers a standardized, efficient method for performing this analysis at scale.

Methods

AI models for tubule detection and classification were developed using 65 whole slide images (WSIs) of only H&E-stained testes from sexually mature beagle dogs. To ensure robust model performance, control and treated dogs with diagnosed testicular findings were included. Two toxicologic pathologists provided labels for the model training and evaluation. 10 WSIs (7 control, 3 treated) were held out as an evaluation set. The classification model assigned each detected tubule to the partially pooled stages: stage 1/2, 3, 4, 5, 6/7, and 8, and additional categories: “artifact”, “tangential”, and “abnormal”. Additionally, the tubule detection model output was used to assess tubular dilatation.

Results

The classification model performed on par with current research (0.87 F1 score, 0.87 precision, 0.88 recall). The model performed similarly across control and treated groups and generalized across varying staining intensities and tissue qualities. Application of the models showed significant differences between control and treated cases with increased tubular dilatation and a higher share of “abnormal” tubules.

Conclusion

This AI approach quantifies spermatogenic stages, artifacts, tangential and abnormal tubules in canine testes, and provides standardized assessment of useful metrics such as tubular diameter. This rapid, unbiased and scalable approach has the potential to significantly enhance testicular toxicity evaluation in non-clinical studies. In the future the development of comparable open source models could significantly facilitate toxicological assessments.

POSTER PRESENTATIONS

P36 | Female Reproductive System Changes in Sprague-Dawley Rats Following Intraperitoneal Injection of Sesame Oil as Vehicle in Dual Route Studies

Anikethana Ramesh, Rajesh Karunanithi, Manohar Madhav Deshmukh, Kiran Hosaholalu Jayaram, Srinivasa Prahalada, Mohan Krishnappa, Kalaiselvan Ponnusamy
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Introduction

Pre-clinical evaluation of certain medical devices requires administration of test item extract through intravenous (IV) and intraperitoneal (IP) routes. This study summarizes the microscopic changes noted in female reproductive system of Sprague-Dawley rats from 30-day dual route IV/IP studies in comparison with IV only studies.

Methods

Data was collected from 284 females aged 12–16 weeks across 11 studies for IV/IP route and 280 females aged 12–16 weeks across 10 studies for IV route. Vehicle for test item extract preparation for IV and IP studies were normal saline and sesame oil, respectively.

Results

In dual route IV/IP studies, microscopic changes included decidual reaction (9.85%) in uterus, vaginal mucification (7.04%), mammary gland hyperplasia (12.67%), uterine endometrial folding (3.16%) and cervical mucification (2.11%).
In I/V only studies, microscopic changes included a single incidence of decidual reaction (0.35%) in uterus, vaginal mucification (2.5%), mammary gland hyperplasia (3.2%), uterine endometrial folding (0.7%) and cervical mucification (0.7%).

Conclusion

The increased incidences of uterine decidual reaction and related changes in IV/IP studies in vehicle control and test item groups suggest the role of sesame oil. As per literature, intrauterine instillation of sesame oil results in induction of deciduoma in rats. Sesame oil injected in the peritoneal cavity migrated to the uterine lumen through the ovarian bursa and fallopian tube. Repeated restraining of animals for test item extract administration may also lead to pseudopregnancy, which in turn may predispose to decidual reactions. Hence, the changes in female reproductive organs were primarily attributed to sesame oil in dual route studies.

POSTER PRESENTATIONS

P37 | Self-Supervised Feature Learning and Clustering for Unsupervised Anomaly Detection in Toxicologic Pathology

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Introduction

Toxicologic histopathology entails the identification of anomalies outside the control data distribution. In addition to identifying known anomalies, pathologists must also identify rare or novel anomalies. Due to the unknown number of anomalous classes and the difficulty of scaling the training data to include all possible tissue anomalies, the traditional supervised computational models are bottlenecked. We present an unsupervised learning method for identifying anomalous regions in Wistar Rat whole-slide H&E-stained images.

Methods

We employ this method to liver and kidney tissues, but it is applicable to other organs as well. The training data consists of 100 (50 kidney and 50 liver) control images. Test Data consists of 10 WSIs (five normal and five abnormal) for each organ.

A features extractor is trained using self-supervised learning (SSL), with a patch dimension of 224x224 extracted from 50 WSIs at 20x magnification. Approximately one million tiles were used to train the model. The SSL-trained feature extractor generates feature representations for all training patches, which are clustered to derive sub-types of normal tissue. A heatmap depicting the probability of anomalous tissue present is generated for pathologist interpretation.

Results

Using ground truth with pathologists, the method achieves an AUC of 82~85 percent for Liver and Kidney in identifying the anomalous regions. Pathologists reported a high sensitivity, which enabled the identification of most anomalous regions.

Conclusion

The presented proof-of-concept study enables automated anomaly detection without the need for annotated data. The model is designed to pick unseen abnormalities, making it an effective tool for pathologists reporting.

POSTER PRESENTATIONS

P38 | CystAssist: Automated AI quantification of kidney features in preclinical murine model of ADPKD

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common life-threatening genetic diseases, affecting 1 in 400-1000 people. We have developed an automated tool to segment and quantify kidney features in a murine model of ADPKD.

Methods

We used a juvenile cystic kidneys mouse model: a recessive mutation in the mouse which causes the development of polycystic kidney disease. To examine the relationship between disease progression by age and cystic index readout in mice, we collected kidneys at the age of 5-, 8-, 10-, 12-, 14-weeks. Kidneys were sectioned at 5 µm, stained with hematoxylin and eosin, and scanned with an Aperio whole slide scanner at 40x magnification. 58 sections and corresponding pathologist-based cyst annotations were used for training a neural network to detect cystic regions, papilla, and fat in each kidney; 32 sections were used for validation.

Results

To validate the performance of this algorithm, kidney segmentations were reviewed and corrected manually by a pathologist. In this analysis, the CystAssist segmentation algorithm scored an average sensitivity of 0.92 and specificity 0.99.

Conclusion

We have developed a platform to precisely quantify kidney features. This tool saves the pathologist valuable work time, allowing them to concentrate on more difficult, scientifically challenging, and interesting tasks. The same platform can segment and phenotype glomeruli and tubules, and enumerate podocytes, forming a comprehensive platform for evaluating kidney tissue in health and disease. The segmentation tool described utilizes the open-source work: https://github.com/SarderLab/Histo-cloud.

POSTER PRESENTATIONS

P39 | Efficiency Study to Evaluate Use of AI-Based Decision Support Tool for Toxicological Pathology

Laoise Lord Bissett[1], Lise Bertrand[2], Jogile Kuklyte[1], Eoghan Keany[1], Daniel Rudmann[2], Shane Ryan[1], Colin Doolan[1], Donal O'Shea[1], Mark Gregson[1]

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Introduction

An AI-based decision support tool (AI-DST) was developed to aid pathologists in the digital slide review workflow. The tool provides information on the distribution and severity of several common lesions in 5 potential target organs in the rat. The goal of this study was to compare the pathologist's experience in a digital pathology diagnostic workflow with and without AI-based decision support tools.

Methods

Twelve board certified toxicologic pathologists were provided de-identified study material that had the AI-DST classifiers available. A survey was completed by each pathologist to assess the impact of the AI-DST on target organ identification, lesion recognition, and to confirm the value of the AI-DST as a second opinion by confirming or updating the findings and targets reported previously

Results

All pathologists made changes to their findings based on use of AI. Pathologists found the AI-DST had a positive impact on confirming findings, localising small lesions and subtle changes, identifying abnormal areas, and grading lesions. However, the effectiveness of the AI-DST varied depending on the organ being reviewed due to false positive detection, which could distract the pathologist's review. Pathologist feedback was included for targeted improvements of the tool.

Conclusion

The AI-DST helps the study review workflow to be more efficient and consistent. The tool is designed to be used as a support tool, whereby pathologists use the output similar to when they consult a professional colleague, or literature reference. The improvements identified during the study will be addressed for further generalisability of the AI-DST.

POSTER PRESENTATIONS

P40 | INHAND: International Harmonization of Nomenclature and Diagnostic Criteria for Lesions - An Update - 2023

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Introduction

The INHAND Proposal (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) has been operational since 2005. A Global Editorial Steering Committee (GESC) coordinates objectives of the project. Development of terminology for rodent organ systems or non-rodent species is the responsibility of Working Groups, with experts from North America, Europe, and Japan. All rodent organ systems have been published - Respiratory, Hepatobiliary, Urinary, Nervous Systems, Male Reproductive and Mammary, Zymbals, Clitoral and Preputial Glands and Hematolymphoid System in Toxicologic Pathology and the Integument and Soft Tissue, Female Reproductive System, Digestive System, Cardiovascular System, Skeletal System, Special Senses and Endocrine System in the Journal of Toxicologic Pathology as supplements and on a web site - www.goReni.org. Mini-pig and Dog have been published in Toxicologic Pathology in 2021 and Non-human primate and Rabbit have been published in the Journal of Toxicologic Pathology in 2021. Fish and Non-rodent ocular toxicity group are targeted for manuscript review in 2023. INHAND guides offer terminology, diagnostic criteria, differential diagnoses, images, and guidelines for recording lesions in toxicity and carcinogenicity studies. INHAND GESC representatives work with representatives of FDA Center for Drug Evaluation and Research (CDER), Clinical Data Interchange Standards Consortium (CDISC), and National Cancer Institute (NCI) Enterprise Vocabulary Services (EVS) to incorporate INHAND terminology as preferred terminology for SEND (Standard for Exchange of Nonclinical Data) submissions to the FDA. Interest in INHAND nomenclature, based on input from industry and government scientists, is encouraging wide acceptance of this nomenclature.

Methods

NA

Results

NA

Conclusion

NA

POSTER PRESENTATIONS

P41 | Investigation of deep learning using nuclear segmentation Methods to detect rat hepatocellular hypertrophy

Jogile Kuklyte[1], Esther Crouch[2], Laoise Lord Bissett[1], Lise Bertrand[2], Daniel Rudmann[2], Shane Ryan[1], Colin Doolan[1], Donal O'Shea[1], Mark Gregson[1], Hope Williams

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Introduction

Hepatocellular hypertrophy commonly occurs following exposure to xenobiotics and may be challenging to diagnose consistently, especially when panlobular. Hepatocyte size can be estimated by counting the number of nuclei in a defined area. Nuclear counts on the overall slide can be represented using a density map. AI-enabled nuclear segmentation facilitates density-based mapping of hepatocytes as a surrogate for cell size changes and directs pathologists to potential hypertrophic lesions at a whole slide view.

Methods

Two nuclear segmentation classifiers were investigated; a generic semantic nuclei segmentation classifier and a pixel instance hepatocyte nuclei segmentation classifier. Filters were calculated to counteract over-segmentation of nuclei that produced false negatives. Evaluation used density maps (hypertrophic and non-hypertrophic regions, highlighted by pathologists) to assess the correlation between hypertrophy and nuclear density.

Results

Filtering must be used to achieve sufficient performance of the nuclear segmentation classifier. Filtering improved performance, with decreased nuclear density in hypertrophic regions, although some overlap with normal regions was seen. Further refinement of filtering capabilities could be helpful for isolating zonal and sex-based differences in hypertrophy detection.

Conclusion

Nuclear segmentation density maps have the potential to be used to aid hypertrophy detection. Challenges include zonal differences of hepatocyte size and other study-specific factors (sex and age of animal). Consideration of these will assist in improving the accuracy of hypertrophy identification in rat liver.

POSTER PRESENTATIONS

P42 | Deep Learning for Artefact Identification and Quantification in Digital Pathology

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Introduction

Artefacts from fixation, processing, sectioning, and scanning can degrade whole slide images (WSIs) of histopathology specimens. WSI artefacts can make histopathology slides unsuitable for review and automatic analysis. Manually checking slides for artefacts in preanalytical procedures is time-consuming and resource-intensive, increasing costs and production times. We describe a system trained to identify and quantify frequently occurring artefacts in WSI of H&E-stained tissues.

Methods

The training set consisted of 9255 H&E WSIs from varied tissue types, species, and stain quality obtained with a Hamamatsu Nanozoomer XR. The test set included 100 WSIs from rats and dogs obtained with Hamamatsu XR and Aperio GT450 scanners and having scanning and processing artefacts. A multi-magnification deep learning system identifies and quantifies nine artefacts: out-of-focus regions, air bubbles, tissue folds, pen traces, knife lines, stain deposits, dust, no tissue, and coverslip lines. The system uses nonlinear features to categorise and segment artefacts.

Results

This study's analysis is ongoing. A Histo-technician analyse the results qualitatively using the system's segmentation mask. The study of a subset of open TG-Gates data (liver and kidney tissue) on 3166 WSIs showed a precision of 0.84~0.93 and a recall of 0.89~0.96 for all artefact categories. The segmentation mask's F1-score ranged from 81 to 92%, depending on the artefact. The algorithm executes in less than 15 seconds for a 1GB image.

Conclusion

The system integrates an automated quality control system into the preanalytical workflow in histopathology to quickly identify slides that need to be reprocessed, or rescanned. By excluding regions from training and testing, it can help develop more generalized AI algorithms.

POSTER PRESENTATIONS

P43 | Tissue Identification in Histopathology Images for Workflows in Nonclinical Toxicologic Pathology

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Introduction

Automated image analysis requires segmentation of individual sections on multi-organ slides. Identifying tissue sections allows a quality control check against the anticipated list of tissue based on the study design and creates metadata for tissue-specific analysis algorithms and content-based image retrieval systems (CBIR). We present a Deep Learning (DL) approach for organ-specific anatomical classification of Whole Slide Image (WSI) from H&E-stained tissue sections of Wistar rats.

Methods

Training dataset made up of 2,880 WSIs featuring 8,166 organ ROIs from eight laboratories scanned with a Hamamatsu XR. The test set consisted of 1000 WSIs acquired with a Hamamatsu XR and Aperio GT450 scanners. Morphological features from two magnifications are used for tissue classification.
A high-magnification self-supervised learning (SSL) model extracts spatial context and cellular information to complement a low-magnification model that extracts shape, size, and colour features. A multitask DL network classifies and segments tissue boundaries using the feature set.

Results

This study's analysis is ongoing. The model's segmentation mask is used by subject matter experts to evaluate the findings qualitatively. Quantitatively, on a public dataset [1] of 320 WSIs, the algorithm identified 27 organs with 94% accuracy and 93% recall.
[1] MMO-Net, Journal of Pathology Informatics 13 (2022): 100126.

Conclusion

The proposed model classifies organs objectively, precisely, and accurately. The organ identification model is anticipated to be beneficial for preanalytical quality control to validate laboratory information system metadata and as a preprocessing phase for subsequent image analysis tasks.

POSTER PRESENTATIONS

P44 | Detection of mRNA in formalin-fixed paraffin-embedded cochlea from monkeys using in situ hybridization (ISH) techniques

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Introduction

Toxicity and biodistribution of gene therapy products must be evaluated. While PCR techniques remain the gold standard to quantifying vector DNA / transgenic RNAs, ISH techniques have the advantage of providing a morphological context but are often considered as challenging to develop, poorly sensitive and not quantitative, especially in tissues difficult to process such as the cochlea.

Methods

Six non-human primates received a single intracochlear injection of an AAV vector engineered for the treatment of a specific condition leading to hearing loss and were terminated after a 3-month recovery period. The cochlea were sampled, fixed, decalcified, paraffin-embedded and processed to slides. Detection of endogenous and transgenic mRNAs were attempted by fluorescence using the RNAscope technology.

Results

The mRNAs of interest were localized in the target cells as expected, providing valuable information. Several parameters had to be optimized, including the decalcification step, the ISH protocol, slide mounting and image acquisition, especially to adjust for tissue autofluorescence.
ISH assays can be performed on fresh frozen, fixed frozen and FFPE samples. Formalin and paraffin embedding, while providing excellent morphology, may cause up to 30% of RNA loss; which is detrimental to sensitivity. These drawbacks were addressed using the RNAscope technology; which is compatible with partially degraded RNAs and stands out for its high sensitivity.

Conclusion

RNAscope labeling allows localization, which is complementary to the in-solution genomic approaches, quantification if combined to image analysis, allows to visualize multiple transcripts simultaneously, and could be combined with IHC in order to visualized RNA and proteins in the same section.

POSTER PRESENTATIONS

P45 | Comparison of two methods (ISH versus IHC) to detect human cells in murine tissue sections

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Introduction

Before being marketed, safety and efficacy of any cell therapy must be assessed. Biodistribution and persistence of therapeutic cells can be investigated by sensitive molecular biology techniques but also by IHC or ISH assays that provide morphological context. The performance of such markers in FFPE tissues were compared in a toxicity/biodistribution study where human therapeutic cells were administered to NSG mice. More specifically, two markers were compared, including sensitivity, specificity, ease of use and cost.

Methods

32 mice were injected intravenously 0 or 5.106 human therapeutic cells developed to regulate the immune system. Mice were euthanized on Days 7 and 28 and several organs investigated for the presence of therapeutic cells using IHC or ISH markers (KU80 and ALU, respectively). All slides were evaluated histologically, digitized, and analyzed (Visiopharm software). The total number of positive cells was counted.

Results

On Day 7, both ALU and KU80 were relevant in labelling the human cells in the lungs, whereas ALU was better on Day 28 to detect rare events. Image analysis confirmed that ALU hybridization was more sensitive and specific than KU80 immunolabeling.

Conclusion

In conclusion, KU80 and ALU detection were both helpful in assessing the limited persistence of human cells in mice over time. The choice of the marker depends on the number of cells, the quantification strategy, and the cost.

POSTER PRESENTATIONS

P46 | A GLP-Compliant Digital Pathology Workflow Solution for Nonclinical Safety Assessment Studies

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Introduction

Regulatory acceptance, data storage and distribution performance issues, lack of system integration, quality control, and image retrieval/retention services hamper digital pathology (DP) implementation. We built a comprehensive DP solution for preclinical anatomic pathology evaluation and show the efficacy on a GLP-compliant safety study.

Methods

We designed a whole slide image viewer for assessing, annotating, and reporting. A GLP-compliant nonclinical safety study in rodents was used to validate the system by qualifying the slide scanner, scanner software, data repository & image management software, viewer, transfer protocol, and hardware. Board-certified pathologists assessed the workflow and WSIs' equivalency with glass slides.

Results

Three pathologists evaluated two hundred images and verified that the system reproduced tissue slides accurately. The accuracy of each annotation (length and area measurement) displayed on the viewer was within 5% of the reference value. Comparable results were obtained for all human factor testing (reviewing and reporting) between pathologists utilising the system and glass slides. Using this system, the pathologists were able to complete the study a week earlier than if they had relied on the traditional glass slide-based technique. Using the solution resulted in the delivery of an anatomic pathology report with the same findings as when pathologists reported using glass slides, validating the workflow's Fit-for-purpose.

Conclusion

A GLP-compliant digital pathology workflow solution for nonclinical safety assessment is shown. The system has the potential to enhance preclinical anatomic pathology reporting and help expedite DP adoption.

POSTER PRESENTATIONS

P47 | Artificial intelligence-based differential counting of rat ovarian follicles in hematoxylin-eosin stained histological images

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Introduction

Differential ovarian follicle counting is one of the tests that aim to estimate potential reproductive toxicity. Manual counting of ovarian follicles from histological tissue sections has been a standard method, but it is time-consuming and prone to intra- and interobserver variability.

Methods

168 hematoxylin-eosin (HE) stained rat ovarian tissue sections from two different laboratories were used for training an AI model using convolutional neural networks. The AI model was trained to detect four classes of ovarian follicles: primordial, primary, growing, and antral, by supervised object detection training. Only nucleated follicles were included, to avoid double detection when using step-sections. The AI model performance was compared against annotations by two independent veterinary pathologists. The pathologists annotated a total of 31 regions of interest of approximately 0.5 mm2 each, across 16 whole-slide images.

Results

The F1 score for Pathologist 1 vs Pathologist 2 was 91,3%; Pathologist 1 vs AI 90,86%; and Pathologist 2 vs AI 91,36%. Most errors, both between pathologists as well as by the AI model, came from misclassification into a different class (e.g. primary instead of primordial). Agreement was highest for primordial and primary follicles.

Conclusion

AI-based quantification of ovarian follicles from digital whole-slide images of HE-stained sections performed comparably to standard manual counting by pathologists. Consistent quantification and classification of ovarian follicles is challenging and time-consuming for pathologists, but AI-based assessment can both expedite and increase results consistency between animals and studies. A GLP validated system enables the use of the classifier in regulatory studies.

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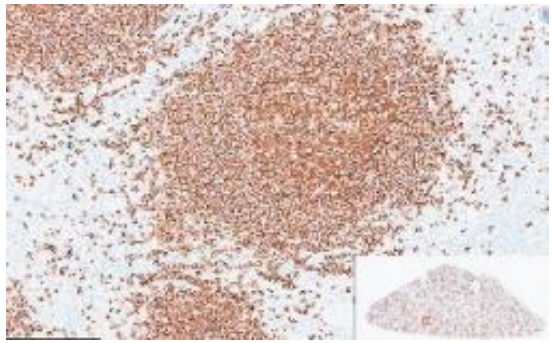


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28 -31 AUGUST 2024
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The European Society of Toxicologic Pathology, the European Society of Veterinary Pathology & The European College of Veterinary Pathologists are pleased to invite you to the 5th Cutting Edge Pathology Congress. This congress is special as the conference will be a joint venture between the European Society of Toxicologic Pathology, the European Society of Veterinary Pathology and European College of Veterinary Pathologists with an extensive program covering veterinary pathology as well as toxicologic pathology. The meeting will enable both disciplines of pathology to learn from each other's work and we hope it provides opportunities for new collaborative ventures between veterinary and toxicologic pathologists.

For the first time, **5th Cutting Edge Pathology Congress** will be held in San Lorenzo de El Escorial, the mountainous northern satellite town close to Madrid at the Real Centro Universitario Maria Cristina from 28th to 31st August 2024. The venue of the congress is part of the historic monastery declared a UNESCO World Heritage Site and location of the summer courses of the University Complutense of Madrid. Tucked into the mountainous surrounding, San Lorenzo de El Escorial offers not only the impressive monastery closely linked to the Spanish crown, but also quaint little boutiques and a collection of vibrant Spanish tapas bars and elegant restaurants. However, this shall not distract from this event intended to provide a forum for colleagues working in toxicologic and veterinary pathology not only from Europe but from all over the world coming together to exchange their expertise, current challenges and future visions on all aspects of Cutting Edge Pathology.

Two Scientific Committees (ESVP/ECVP and ESTP), comprising both nationally and internationally recognized experts are responsible for preparing the scientific program of the congress.The ESTP part of the congress will be held under the fascinating topic "Neuropathology- The Vast Pink Wonderland". This in an incredibly complex field, encompassing both preclinical and clinical aspects of assessment and therapy. Neurodegenerative diseases serve as a prime example of how impactful neuropathology can be in our daily lives, not to mention the renewed interest in CNS diseases like Alzheimer's, since a number of new drugs received marketing authorization last year. The program will also include additional side topics of highly relevant interest for toxicologic pathologists and preclinical safety scientists. There will be plenary lectures, oral presentations of original scientific work, interactive case presentations, and poster session. In addition to interesting ESVP/ECVP/ESTP Joint Plenary Lectures, the ESVP/ECVP part of the congress will offer a wide range of updated veterinary pathology topic sessions, covering diseases in both domestic and non-domestic animals. Engaging interactive workshops and educational sessions will be held, along with poster presentations and oral communications. Importantly, the top posters and oral communications will be eligible for prizes to recognize their outstanding contributions to the field. This congress will provide an excellent opportunity to meet and exchange science and knowledge with veterinary pathologist colleagues from all over Europe. Furthermore, following tradition, we will give a warm and proud welcome to the new ECVP graduates of 2024, who represent the brightest future of our specialty.

During the congress you will dive into cutting edge scientific content and also an international trade exhibition where contract laboratories and other services display books, new equipment and latest technologies, etc. in the halls of the Real Centro Universitario Maria Cristina. For all participants, the social program will include a welcome reception and a congress dinner español.

We would be very pleased to welcome you to San Lorenzo de El Escorial (Madrid) in August 2024!