

Welcome

Dear Colleagues, Friends, and Guests,

On behalf of the European Society of Toxicologic Pathology (ESTP) and the Italian Society of Toxicologic and Experimental Pathology (SIPTS), it is our honour and pleasure to welcome you to the 10th EUROPEAN CONGRESS OF TOXICOLOGIC PATHOLOGY in Stresa, Italy.

The congress Organizing Committees (Scientific and Local) have planned an excellent program on Toxicopathology of the Soft Tissue and Musculo-Skeletal System for the first days, and an additional special session on Friday 14 September focused on topics of special interest for the toxicopathology profession.

The sessions on "Toxicopathology of the Soft Tissue and Musculo-Skeletal System" will include lectures addressing fundamentals of bone pathophysiology, presentations on animal models of bone disease, including osteoporosis, osteoarthritis, cancer bone metastasis, muscular dystrophy. A special lecture will address the current perspectives of stem-cell based therapy for Duchenne Muscular Dystrophy with examples of preclinical to clinical translation. Additional presentations will offer examples of in vivo imaging approaches to the evaluation of bone and muscle in the context of preclinical studies in animal models, an overview of clinical pathology and biomarkers for the musculo-skeletal system, examples of compound-induced lesions in bone and soft tissues, and evaluation of devices and biomaterials in experimental in vitro and in vivo models with examples of clinical translation, as well an overview on the preclinical safety evaluation of devices and biomaterials.

The closing session of Friday 14 will focus on "Topics and issues in toxicopathology work and practice" and will address themes of critical importance for all colleagues involved in preclinical safety assessment. This session will include a summary of global regulations applicable to pathology endpoints in repeat dose studies, a presentation on the current international perspectives, recommendations and issues with Pathology Peer Review in preclinical safety studies, an overview on the definition and application of Pathology Working Groups (PWGs) as problem-solving tools for preclinical safety studies with examples, presentations outlining issues, perspectives and recommendations on interactions between Sponsor's and CRO's pathologists.

A most interesting round table discussion on "The Future of Toxicologic Pathology" will take place in the late afternoon of Thursday 13 September. This will be an opportunity for the audience to discuss themes of major relevance to the toxicopathology profession in the context of a challenging global situation for pharma/biotech/chemical R&D and for the "preclinical outsourcing" market.

We have organized some pleasant social events for you. On Tuesday evening we would like to invite you to join the welcome reception at the sky bar of the Hotel la Palma. From here you will have a beautiful view over the city of Stresa and the Lake Maggiore.

The congress dinner will take place at Thursday evening on one of the beautiful Islands in the Lake Maggiore. Please join us and use the unformal evening to meet old and new friends and colleagues. The dinner is planned as a walking dinner. You will have a free choice between different restaurants and can move from course to course. This gives you the opportunity to meet different people at the stations and socialise with everyone.

And please do not miss the Annual General Assembly of the ESTP. The assembly of the ESTP membership will take place on Wednesday 12 September from 17.30 to 19.30 in the main session room at the Palazzo dei Congressi. The results of the elections in the Executive Committee board will be presented, and a discussion with the audience on the future Journal strategy for the society will be addressed.

Exhibitors offering services and products related to the toxicopathology profession are a very important part of as well as a precious support for our meeting. Please take the time to visit their booths during the congress.

We look very much forward to meeting you during the week and we wish you to enjoy both the congress and Stresa.

On behalf of the Local and Scientific Organizing Committees

Michela Carbonatto, Wolfgang Kaufmann & Francesco Marchesi

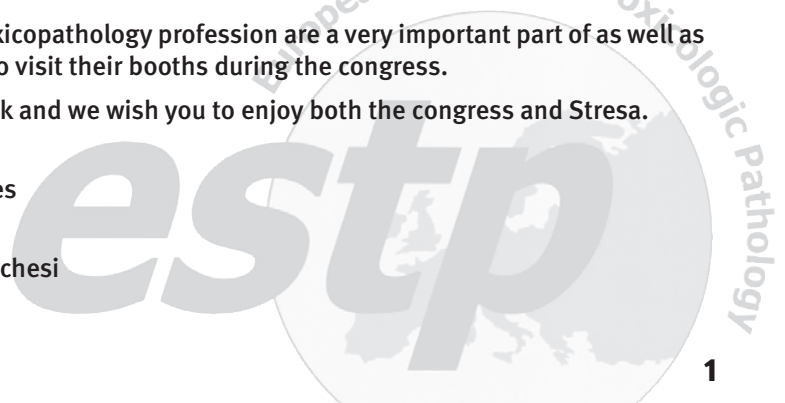


Table of Contents

Welcome	1
Table of Contents	2
General Information.....	7
Local Organizing Committee	7
Congress Organizers.....	7
Scientific Organizing Committee.....	7
Congress Venue.....	8
Accessibility for Persons with Disability	8
Currency.....	8
How to get to Stresa... ..	8
Climate.....	9
Registration Desk	9
Speaker Information.....	9
Poster Presentation	9
Language.....	9
Internet Access	9
Messages.....	10
Congress Bags.....	10
Gastronomy	10
Safety and Security.....	10
Emergency Calls	10
Additional Meetings	10
ESTP Slide Seminar.....	11
Congress CD-ROM.....	11
Abstract Publication	11
Awards.....	11
Social Events.....	12
Industry Exhibition.....	12
Exhibition Quiz	12
Thanks to our Exhibitors	14
Thanks to our Sponsors	15



Table of Contents

Congress Program	16
11 th September, Tuesday	16
12 th September, Wednesday	17
13 th September, Thursday	19
14 th September, Friday	21
Speaker Abstracts	22
S01: Bone remodeling: physiology and pathology	22
S02: Animal models for osteoporosis – bone histomorphometric approach	23
S03: From development to joint disease: animal models of osteoarthritis	26
S04: Mouse models of bone metastasis: pathogenesis and treatment	27
S05: In vivo imaging approaches for skeletal muscle & correlations with histopathology endpoints	30
Satellite Session: The Beagle dog brain atlas project	31
S06: Bone imaging – from bench to bedside	32
S07: Clinical pathology & biomarkers for bone & muscle in preclinical safety studies	33
S08: Comparative long-term preclinical safety evaluation of two glatiramoid compounds in rats and monkeys, with particular emphasize on the subcutaneous toxicity and related systemic changes	34
S09: Review of rodent bone tumors induced by parathyroid hormone and related peptides	35
S10: Brown adipose tissue and effects on the morphology	36
S11: Preclinical models for devices/biomaterials for the skeletal system and examples of clinical translation	37
S12: Preclinical safety evaluation of devices / biomaterials for the musculo-skeletal system	40
S13: Enabling 3D fabrication technologies to generate cell-instructive porous biomaterials for skeletal regenerative therapies.	41
S14: Histopathological effects of phosphodiesterase type IV (PDE IV) inhibitors on the skeletal system of rodents	42
INHAND 01: Proliferative and non-proliferative lesions of the skeletal system and tooth in rodents	43
Part 1: Neoplastic lesions	43
Part 2: Non-neoplastic lesions	44
INHAND 02: INHAND Update – Mammary gland	45

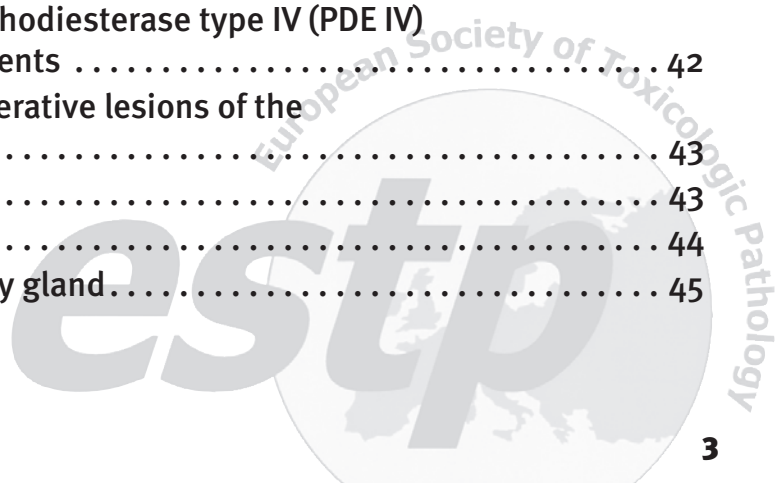


Table of Contents

Case Presentations	46
Case 1: Francesco Marchesi, Elena Riccardi and Anna Maria Giusti	46
Case 2: Céline Thuilliez.	47
Case 3: Elena Riccardi.	48
Case 4: Anna Maria Giusti, Elena Riccardi	49
Case 5: Dirk Nehrbass, Arens, D., Zeiter S.	50
Case 6: Enrico Radaelli, Morvarid Farhang Ghahremani, Michele Ardizzone	51
Case 7: Vittoria Castiglioni, Alessandra Rustighi, Eugenio Scanziani, Enrico Radaelli.	52
Case 8: Anna Lanzoni	53
Speaker Abstracts	54
S15: Animal models for Duchenne muscular dystrophy. From pathogenesis to therapy.	54
S16: Stem cell-based therapy for DMD: current clinical experimentation and novel pre-clinical strategies	57
S17: Pathology endpoints in routine repeated dose toxicology studies: A review of global regulations	58
S18: Pathology Peer Review in preclinical safety studies – international perspectives, recommendations, regulatory issues	59
S19: Peer Review in Toxicologic Pathology – current situation in Japan	60
S20: Pathology Working Groups (PWGs): definition, contexts of application in toxicity and carcinogenicity studies, and examples	61
S21: Interactions between Sponsors and CRO pathologists: What can be improved?	62
S22: Industry-CROs Pathology interactions – a sponsor’s perspective.	63
INHAND Poster	64
INHAND Poster 1: INHAND Update (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice).	64
INHAND Poster 2: Proposed Bone Nomenclature	65
INHAND Poster 3: Proposed Joint and Tooth Nomenclature	66

Table of Contents

Poster Abstracts	67
Po1: Safety of Oncolytic Measles-Virus-SuperCD in Transgenic Mice and Rhesus Macaque	67
Po2: When a biologics drug targets a viral disease: From TCR validation to off-target staining	68
Po3: Evaluation of an antibody drug conjugate (ADC) biodistribution in a selection of mouse tissues using an immunohistochemistry method	69
Po4: Quinolone-induced cartilage changes - an old story in young animals?	70
Po5: A proposal for alternative trimming and inclusion procedure of the knee joint of rodents in toxicity studies	71
Po6: Collagen induced arthritis in the common marmosets: A new non-human primate model for chronic arthritis	72
Po7: Spontaneous metastatic osteosarcoma in control Sprague-Dawley rats from a 2-year carcinogenicity study – three cases	73
Po8: Background pathology of cynomolgus monkeys (<i>Macaca fascicularis</i>) from different origin (Mauritius vs. China/Vietnam)	74
Po9: Workflow for establishing historical control data in the RITA database	75
P10: Digital Image Microscopy Review of Chronic Study PWGs by Pathologists Naïve to the Original Glass Slide Review	76
P11: The Diagnostic Accuracy of Digital Histopathology: Experiences at the NTP and NCTR	77
P12: Quantitative Digital Pathology in Preclinical Safety Studies	78
P13: Automated Digital Morphometry of Thyroid Gland Follicles Using Standard H&E-Stained Tissue Sections and Comparison to Manual Morphometry ...	79
P14: An Experimental Dog Model of Prostate Cancer for In Vivo Imaging and Therapeutic Research	80
P15: Immunohistochemical characterization of proliferative lung lesions in SP-C/c-raf-1-BxB mice	81
P16: Histopathology confirmed the specific uptake of DFMT (O-[¹⁸ F]fluoromethyl D-tyrosine) in tumor metastasis but not in inflammatory infiltrates	82
P17: Subcutaneous injection sites: is the quality of the diagnosis improved when more than one tissue section is examined?	83
P18: Set up a method for linear radiographic measurement of the longitudinal tibial axis in Sprague Dawley rats	84

Table of Contents

P19: Serial Blood Sampling for Dried Blood Spot Technique in Toxicology Studies: Impact on Corticosteron, Histopathology and Clinical Pathology in Crl:CD (SD) Rats	85
P20: Simulated Tail Venipuncture Transiently Increases Serum Cardiac Troponin I . . .	86
P21: Could miRNA-126 become a translational biomarker of vascular injury?	87
P22: Adefovir- and Tenofovir-related renal changes from 28-day oral investigation on Sprague-Dawley rat: histopathology, electronic microscopy, genomics and urinary kidney biomarkers end-points	88
P23: Identification of <i>in vivo</i> biomarkers for vancomycin-induced nephrotoxicity . . .	89
P24: Intermittent Co-administration of a Gamma Secretase Inhibitor with Dexamethasone Mitigates Intestinal Goblet Cell Hyperplasia in Rats	90
P25: Lack of Oral Toxicity in 90-Day Study of the Food Additive, Gum Ghatti	91
P26: Expression of epithelial cell adhesion molecule and proliferating cell nuclear antigen in diethylnitrosamine-induced hepatocarcinogenesis	92
P27: Evaluation of cardiotoxicity induced by peroxisome proliferator-activated receptor gamma agonists in mice	93
P28: Comparative <i>in vivo</i> study of porous bioceramics for osteoconductive and osteoinductive response	94
P29: Toxicity Studies of an Agrochemical Fungicide WP Formulation (Cymoxanil 6% and Propineb 70%) in Sprague- Dawley Rats	95
P30: Vertebral osteomyelitis with spinal cord compression after subcutaneous injection of vaccine in a BALB/c mouse	96
P31: Brain Lesions Associated with Vitamin E Deficiency in Muscovy ducks	97
P32: Induction of gastric atrophy following orally administration of fumonisin B1 in mice	98
P33: Histopathological Study of Acute and Chronic Toxicity Induced by Azole Pesticides in Artemia Urmiana	99
P34: DMBA (7, 12-dimethylbenzanthracene) induces dose dependent multi-organ tumors in BALB/c mice	100

General Information

Local Organizing Committee

Michele Ardizzone Merck KGaA / Merck Serono, Colleretto Giacosa (TO) – Italy
Michela Carbonatto (Chair) Merck KGaA / Merck Serono, Colleretto Giacosa (TO) – Italy
Anna Maria Giusti Accelera S.r.l., Nerviano (MI) – Italy
Wolfgang Kaufmann Merck KGaA / Merck Serono, Darmstadt – Germany
Rosa Anna Manno Research Toxicology Centre (RTC) S.p.A, Pomezia (Rome) – Italy
Francesco Marchesi Accelera S.r.l., Nerviano (MI) – Italy
Cristiano Marozin Merck KGaA / Merck Serono, Colleretto Giacosa (TO) – Italy

Congress Organizers

Pia Schröder
Solution office e. K.
Bergstr. 2
29646 Bispingen
Germany
Phone: +49 5194 – 97 44 90
Fax: +49 5194 – 97 44 94
e-mail: pia.schroeder@solution-office.de

Scientific Organizing Committee

Michele Ardizzone Merck KGaA / Merck Serono, Colleretto Giacosa (TO) – Italy
Fiorella Belpoggi Cesare Maltoni Cancer Research Centre – Ramazzini Institute, Bentivoglio (BO) – Italy
Catherine Botteron Bracco Suisse SA, Geneva, Switzerland
Franck Chanut Glaxo-SmithKline, Ware – United Kingdom
Paul-Georg Germann Nycomed GmbH, Konstanz – Germany
Anna Maria Giusti Accelera S.r.l., Nerviano (MI) – Italy
Wolfgang Kaufmann Merck KGaA / Merck Serono, Darmstadt – Germany
Francesco Marchesi (Chair) Accelera S.r.l., Nerviano (MI) – Italy
Rosa Anna Manno Research Toxicology Centre (RTC) S.p.A, Pomezia (Rome) – Italy
Matthias Rinke Bayer HealthCare / Bayer Pharma AG, Wuppertal – Germany
Nigel Roome Sanofi R&D, Vitry sur Seine – France
Ian Taylor Huntingdon Life Sciences, Eye – United Kingdom
Grazyna Wieczorek Novartis Pharma AG, Basel – Switzerland

General Information

Congress Venue

Palazzo dei Congressi di Stresa
Piazzale Europa, 3
28838 Stresa (VB)
Italy
Phone: +39 0323 30389
Fax: +39 0323 33281

The conference will take place at the "Palazzo Congressi Stresa", the conference centre of the City. The venue is located in walking distance to all offered hotels as well as to the very beautiful centre of Stresa and the Lago Maggiore.

Accessibility for Persons with Disability

Please use the front door.

Currency

The Euro is Italy's currency. For the latest rates, check out www.xe.com.

How to get to Stresa...

By car

From Turin: Take the A4/E64 motorway and follow it in direction to „Alessandria/Gravellona Toce". Take the direction to Gravellona Toce

Follow the A26 motorway in direction to „Gravellona Toce" to the Lake Maggiore: exit in Arona or Baveno/Stresa.

From Milan: Take the A8 motorway and follow it in direction to the A26 „Genova/Gravellona Toce". Follow the A26 motorway in direction to „Gravellona Toce" to the Lake Maggiore: exit in Arona or Baveno/Stresa.

By plane

Airports next to Stresa: the closest airport is Milan Malpensa Airport (45 Km), alternatives can be Milan Linate Airport (110 Km), Milan Bergamo Orio al Serio Airport (130 Km) or Torino Caselle Airport (140 Km).

How to get from Milan Malpensa Airport to Stresa

Option n° 1

You can take a private taxi at the cost of approximate Euro 95,00 (one way) to be paid directly to the driver.

Option n° 2

Take the shuttle bus from Malpensa Airport to Stresa at the cost of Euro 12,00 per person. It can be payed directly to the driver. Seats reservation is required. You have to book it at least one day before arrival. Please visit the homepage of the shuttle service www.safduemila.com, call 0039.0323.552172 or send an e-mail to alibus@safduemila.com.

General Information

Climate

September on the Maggiore Lake often includes gloriously hot days in Stresa followed by cool nights (55 – 75°F / 13 – 24°C). In the latter half of the month, it's even starting to feel quite fall-like, with possible brief rainstorms.

Registration Desk

The desk will be located at the ground floor right in front of the meeting room. All the congress documents can be picked up from the registration desk. An identification badge must be worn to enter all the congress sessions and events. Registration is possible during the whole congress.

Opening hours of registration desk:

11th September 2012 11:00 h – 18:00 h
12th September 2012 08:00 h – 18:00 h
13th September 2012 08:00 h – 18:00 h
14th September 2012 08:00 h – 12:00 h

Speaker Information

Video beamer and PC are available for presentations. Please turn in your presentations at the front desk before your session. Please use CD-ROM, USB stick or comparable format. The use of your own PC is not desired.

Poster Presentation

Posters will be exhibited during the entire Congress. The Poster session is scheduled on Wednesday the 12th September at 15.00 h. It will also be possible to go through the posters during coffee and lunch breaks.

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

The poster boards are kindly provided by



Language

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

Internet Access

A laptop with internet access is provided for service during the business hours.

The internet access is kindly provided by



In case you want to use your own computer, wireless Internet access is also available via WLAN.



General Information

Messages

There is a message board close to the Congress Registration Desk.

Congress Bags

Congress bags were kindly provided by



Gastronomy

Coffee, tea, refreshment beverage and pastries are served during the coffee breaks.

Lunch is provided during the lunch breaks on:

Tuesday, September 11

Wednesday, September 12

Thursday, September 13

One of the coffee breaks is kindly provided by



Safety and Security

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

Congress representatives will respond to any media inquiries.

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

Emergency Calls

12 - Telephone Directory Assistance Number

112 - Carabinieri

113 - Emergency Police Help Number (also ambulance and fire)

115 - Fire Department

116 - A.C.I. (Italian Automobile Club) road assistance

118 - Medical Emergencies

Additional Meetings

ESTP Executive Committee board F2F meeting

The ESTP Executive Committee board F2F meeting will take place on Wednesday 12 September 07:30 – 08:00 AM in the Lobby of Regina Palace Hotel, C.so Umberto I, 33, 28838 Stresa.

ESTP Guideline Committee

The ESTP Guideline Committee F2F meeting will take place on Thursday 13 September 12:30 – 13:00 in the Lobby of Regina Palace Hotel, C.so Umberto I, 33, 28838 Stresa.

ESTP Annual General Assembly

The Annual General Assembly will take place on Wednesday 12 September 17:30 – 19:30 in the main conference room.

General Information

Other additional Meetings

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

ESTP Slide Seminar

An internet slide seminar on different cases of toxicologic pathology is again organized in advance (sponsored by 3DHISTECH KFT.). Case descriptions and scanned slides are available electronically via the ESTP Website www.eurotoxpath.org. The contributors will give presentations of their cases during the congress

Congress CD-ROM

A CD-ROM containing several presentations given at this congress in pdf-format is planned to be handed out to the participants.

The production of the CD-ROM is kindly sponsored by Nycomed GmbH

Abstract Publication

Abstracts of the presentations and posters will be published in the official journal of the ESTP: *Experimental and Toxicologic Pathology* in 2013.

Awards

- Boehringer Ingelheim Award for Thesis
- SFPT Award for Best Poster
- IATP Charles Capen Trainee Award
- IFSTP Trainee Award

Every two years, the ESTP offers an award for an outstanding thesis in the field of Toxicologic Pathology sponsored by Boehringer Ingelheim Pharma GmbH & Co. KG. The best thesis will be honored with 5.000 Euro, the second best by 3.000 Euro and the third best by 2.000 Euro.

The award ceremony is scheduled for Thursday 13th of September 10:15 – 10:35 h after Session 5 “INHAND Update – Interactive Sessions”. Please, participate.

General Information

Social Events

At the evening of **Tuesday the 11th September** we would like to invite you to join us at the **Welcome Reception**. It will take place at the **Sky Bar of the Hotel La Palma** from where you will have an amazing view over the Lago Maggiore and the city of Stresa. You will also have the opportunity to meet colleagues and old friends, to chat and prepare yourself for the following days of the conference.

Hotel la Palma
Lungolago Umberto I, 33
Stresa (VB)
Italia 28838

Conference dinner (September 13th); Please feel invited to the conference dinner on Thursday evening which will take place on the beautiful „Isola Bella“. The island becomes even more attractive at night, when tourists are gone and the smooth light of streetlamps turns it in a living picture that stands out from the lake. This beautiful scenery will create a unique night of bygone times.

The transport to the island and back to the land is of course included.

Industry Exhibition

As in previous years, an exhibition featuring Pharmaceutical and Product Companies, Technical Equipment Companies and Medical Publishers will be held within the same setting as the conference. The entrance is free to those registered to the Conference and registered accompanying persons.

The exhibition will open on Wednesday, September 12, at 10:00 h and will then follow the same schedule as the conference. At September 14 the exhibition will close after the afternoon coffee break.

The industry exhibition provides information about the newest technologies and developments available within our scientific area. The exhibiting companies have a unique possibility to efficiently reach their target customer. The ESTP values the support from exhibitors and believes that the on-site discussion and exchange of experience between exhibitors and the congress participants is of invaluable importance and benefit.

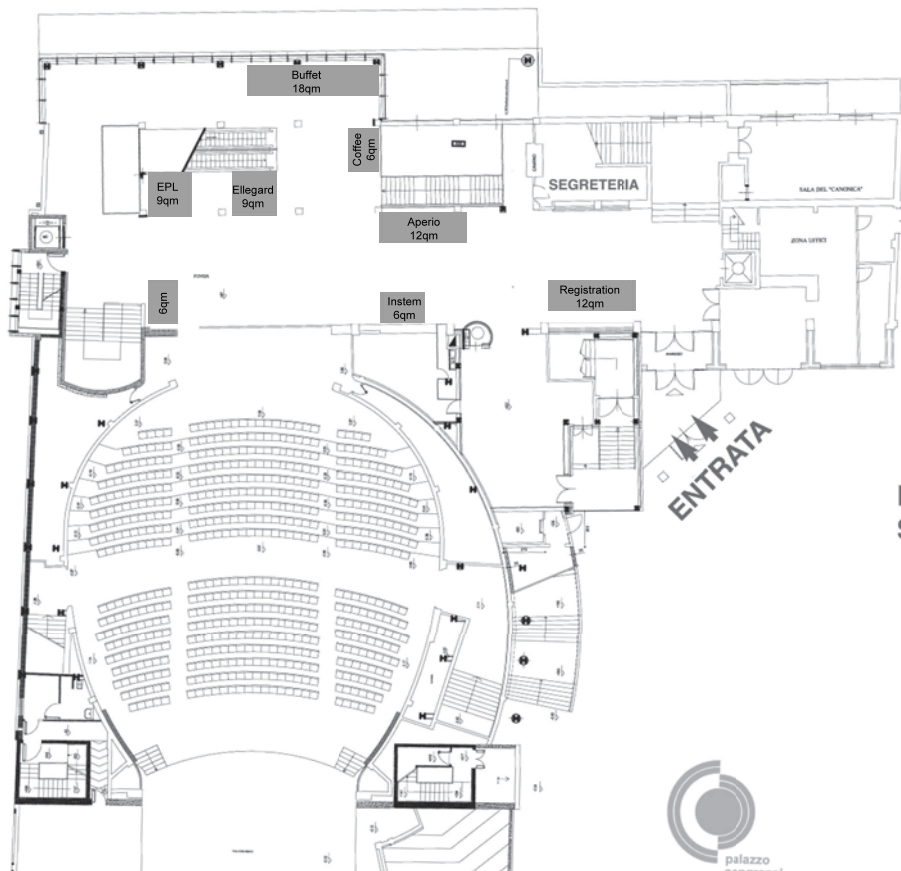
Exhibition Quiz

An exhibition quiz will be performed. The documents needed for your participation will be handed out to you at the congress counter. There will be an attractive prize for the winner. The winner will be awarded on Thursday during the afternoon coffee break.



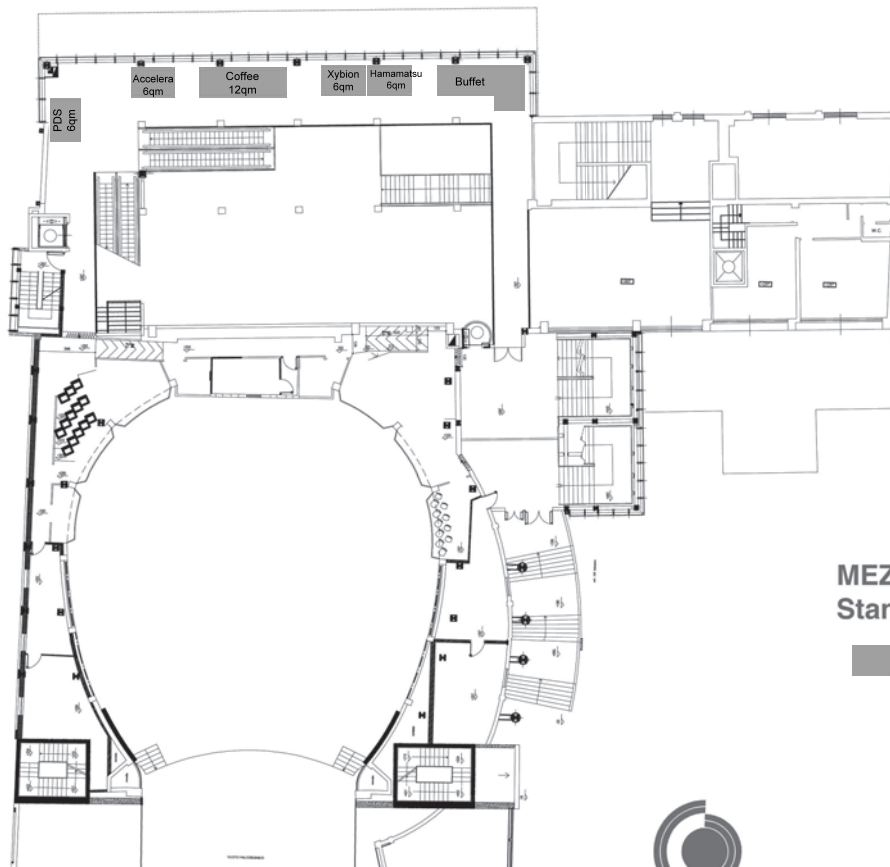
10th European Congress of Toxicologic Pathology

11th September – 14th September 2012 – Stresa, Italy



PIANTERRENO Stand da 1 a 19

■ stand 3x2



MEZZANINO Stand da 20 a 30

■ stand 3x2



General Information

Thanks to our Exhibitors

The ESTP greatly values the support from the following Exhibitors

Accelera	www.accelera.org
Aperio Technologies	www.aperio.com
Ellegaard Göttingen Minipigs A/S	www.minipigs.com
EPL Inc.	www.epl-inc.com
Hamamatsu Photonics	www.hamamatsu.it
Instem LSS	www.instem.com
PDS Ltd	www.pds-europe.com
Xybion	www.xybion.com

General Information

Thanks to our Sponsors

The ESTP greatly values the support from the following Sponsors

BASF SE	www.basf.com
Instem LSS	www.instem.com
LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG	www.lpt-pharm-tox.de
Merck KGaA	www.merckgroup.com
Novartis	www.novartis.com/
Novo Nordisk A/S	www.novonordisk.com
Nycomed GmbH: A Takeda Company	www.nycomed.com
PDS Ltd	www.pds-europe.com
RTC	www.rtc.it
Società Italiana di Patologia Tossicologica e Sperimentale (SIPTS)	

Congress Program

11th September, Tuesday

- 12.00 – 13.45 **Lunch & Registration**
- 13.45 – 14.00 **Welcome Message**
Francesco Marchesi, SOC Chair
- 14.00 – 17.00 **Session 1**
**Musculo-Skeletal System Physiology, Pathology / Physiopathology
of Bone Remodelling / Animal Models of Bone Disease**
Chair: Rosa Anna Manno
Co-chair: Michele Ardizzone
- 14.00 – 14.45 **Bone remodeling: physiology and pathology**
Ermanno Bonucci (Università La Sapienza, Rome, Italy)
- 14.45 – 15.30 **Animal models for osteoporosis – bone histomorphometric approach**
Paola Ballanti (Università La Sapienza, Rome, Italy)
- 15.30 – 16.00 **Coffee Break**
- 16.00 – 16.45 **From development to joint disease: animal models of osteoarthritis**
*Rik Lories (UZ Gasthuisberg / Katholieke Universiteit Leuven Lab for
Skeletal Development and Joint Disorders)*
- 16.45 – 17.30 **Mouse models of bone metastasis: pathogenesis and treatment**
Thomas Rosol (Ohio State University, Columbus, OH, USA)
- 17.30 – 18.15 **Satellite Session – Editorial Focus**
The beagle dog brain atlas project
Xavier Palazzi (Sanofi R&D, Alfortville, France)
- 19.00 – 20.00 **Welcome Reception**



Congress Program

12th September, Wednesday

- 08.15 – 08.30 **Opening Remarks / Introduction**
- 08.30 – 12.00 **Session 2**
In Vivo Imaging Technology Approaches / Clinical Pathology & Biomarkers for the Musculo-Skeletal System
Chair: Grazyna Wieczorek
Co-chair: Ian Taylor
- 08.30 – 09.15 **In vivo imaging approaches for skeletal muscle, & correlations with histopathology endpoints**
Kumar Changani (GSK, Stevenage, UK)
- 09.15 – 10.00 **Bone imaging – from bench to bedside**
Michaela Kneissel (Novartis Pharma AG, Basel, Switzerland)
- 10.00 – 10.30 **Poster Tours & Coffee Break**
- 10.30 – 11.15 **Clinical pathology & biomarkers for the musculo-skeletal system in preclinical safety studies**
David Ledieu (Novartis Pharma AG, Basel, Switzerland)
- 11.15 – 12.00 **Session 3**
Compound-Induced Lesions in Bone, Skeletal Muscle & Soft Tissues
Chair: Ian Taylor
Co-chair: Paul-Georg Germann
- 11.15 – 12.00 **Comparative long-term preclinical safety evaluation of two glatiramoid compounds in rats and monkeys, with particular emphasis on the subcutaneous toxicity and related systemic changes**
Abraham Nyska (Sackler School of Medicine, Tel Aviv University, Israel and RTC Spa, Rome, Italy)
- 12.00 – 13.30 **Lunch & Poster Views**

Congress Program

12th September, Wednesday

- 13.30 – 15.30 **Session 3 (continued)**
Compound-Induced Lesions in Bone, Skeletal Muscle & Soft Tissues
Chair: Ian Taylor
Co-chair: Paul-Georg Germann
- 13.30 – 14.15 **BSTP-sponsored Gopinath Lecture**
Review of rodent bone tumors induced by parathyroid hormone and related peptides
John Vahle (Eli Lilly & Co., Greenfield, IN, USA)
- 14.15 – 15.00 **Brown adipose tissue and effects on the morphology**
Elke Atzpodien (Roche, Basel, Switzerland)
- 15.00 – 15.30 **Poster Tours & Coffee Break**
This coffee break is kindly provided by 
Nycomed: a Takeda Company
- 15.30 – 17.30 **Session 4**
Replacement Therapies (Devices / Biomaterials) for Musculo-Skeletal System
Chair: Francesco Marchesi
Co-chair: Fiorella Belpoggi
- 15.30 – 16.15 **Preclinical models for devices/ biomaterials for the skeletal system and examples of clinical translation**
Milena Fini (Laboratory of Preclinical & Surgical Studies – Rizzoli Orthopaedic Institute, Bologna, Italy)
- 16.15 – 17.00 **Preclinical safety evaluation of devices / biomaterials for the musculo-skeletal system**
Xavier Palazzi (Sanofi R&D, Alfortville, France)
- 17.00 – 17.30 **Enabling 3D fabrication technologies to generate cell-instructive porous biomaterials for skeletal regenerative therapies**
Lorenzo Moroni (MIRA Institute for Biomedical Technology and Technical Medicine Faculty of Science & Technology, University of Twente, the Netherlands)
- 17.30 – 19.30 **ESTP Annual General Assembly**



Congress Program

13th September, Thursday

- 08.30 – 08.45 **Opening Remarks / Introduction**
- 08.45 – 09.15 **Session 3 (continued)**
Compound-Induced Lesions in Bone, Skeletal Muscle & Soft Tissues
Chair: Paul-Georg Germann
Co-chair: Rosa Anna Manno
- 08.45 – 09.15 **Histopathological effects of Phosphodiesterase type IV (PDE IV) inhibitor on the skeletal system of rodents**
Ursula Junker (Novartis Pharma AG, Basel, Switzerland)
Anke Heuser (Nycomed GmbH, Barsbüttel, Germany)
- 09.15 – 10.15 **Session 5**
INHAND Update – Interactive Sessions
Chair: Wolfgang Kaufmann
Co-chair: Catherine Botteron
- INHAND: proliferative and non-proliferative lesions of the skeletal system and tooth in rodents**
Neoplastic lesions:
Heinrich Ernst (Fraunhofer ITEM, Hannover)
Non-neoplastic lesions:
Matthias Rinke (Bayer Pharma AG, Wuppertal, Germany)
- 10.15 – 10.35 **Awards**
- Boehringer Ingelheim Award for Thesis
 - SFPT Award for Best Poster
 - IATP Charles Capen Trainee Award
 - IFSTP Trainee Award
- 10.35 – 11.00 **Poster Tours & Coffee Break**

Congress Program

13th September, Thursday

11.00 – 12.00

Session 5

INHAND Update – Interactive Sessions (continued)

Chair: Wolfgang Kaufmann

Co-chair: Catherine Botteron

INHAND: proliferative and non-proliferative lesions of the mammary gland in rodents

Karin Küttler / Heike Marxfeld (BASF, Ludwigshafen, Germany)

12.00 – 13.30

Lunch & Poster Views

13.30 – 15.00

Interactive Session Case Presentations

Chair: Catherine Botteron

Co-chair: Matthias Rinke

15.00 – 17.30

Session 6

Regenerative / Cell-Based Therapies for Muscle Disease

15.00 – 15.45

Animal models for Duchenne Muscular Dystrophy. From pathogenesis to therapy

Thibaut Larcher (Veterinary School, Nantes, France)

15.45 – 16.15

Poster Tours & Coffee Break

16.15 – 17.00

Stem cell-based therapy for Duchenne Muscular Dystrophy (DMD) – clinical translation

Francesco Saverio Tedesco (Dept. of Cell and Developmental Biology, University College London, London, UK)

17.00 – 18.30

Workshop / Round Table

The Future of Toxicologic Pathology in Europe:

How do Toxicologic Pathologists respond to R&D Downsizing in the European Pharmaceutical Industry?

- Part 1: The Productivity crisis in pharmaceutical R&D
- Part 2: The role of toxicologic pathologists in the biopharmaceutical industry
- Part 3: The role of the ESTP in shaping the future of toxicologic pathology

Moderator: Lars Mecklenburg (Nycomed/Takeda, Barsbittel, Germany)

19.30 onwards

ESTP Congress Dinner

Congress Program

14th September, Friday

- 08.15 – 08.30 **Opening Remarks**
- 08.30 – 12.30 **Session 7**
Topics and Issues in Toxicopathology Work and Practice
Chair: Takanori Harada / Francesco Marchesi
Co-chair: Paul-Georg Germann
- 08.30 – 09.10 **Pathology endpoints in routine repeat dose toxicity studies: a review of global regulations**
Ken Schafer (Vet Path Services Inc., Mason, OH USA)
- 09.10 – 10.00 **Pathology peer review in preclinical safety studies – international perspectives, recommendations, regulatory issues**
Erio Barale-Thomas (Janssen R&D, Beerse, Belgium)
- Peer Review in toxicologic pathology – current situation in Japan**
Takanori Harada (Institute of Environmental Toxicology, Ibaraki, Japan)
- 10.00 – 10.20 **Coffee Break**
- 10.20 – 11.00 **Pathology Working Groups (PWGs): definition, contexts of application in toxicity and carcinogenicity studies, and examples**
Jerry Hardisty (EPL Inc. Research Triangle Park, NC, USA)
- 11.00 – 11.45 **Interactions between Sponsors and CRO pathologists: what can be improved?**
Kathleen Funk (EPL Inc. Sterling, VA, USA)
- Industry-CROs pathology interactions – a Sponsor's perspective**
Armando Irizarry Rovira (Eli Lilly & Co., Indianapolis, IN, USA)
- 11.45 – 12.15 **Additional Audience Q & A session on Side topics:**
- Pathology Endpoints in Repeat Dose Tox Studies
- Pathology Peer Review
- Pathology Working Groups
- Industry-CROs interactions
- 12.15 – 12.30 **Closing Remarks**
Francesco Marchesi & Annette Romeike

Speaker Abstracts

S01: Bone remodeling: physiology and pathology

Ermanno Bonucci
Università La Sapienza, Rome, Italy

Bone is not a static tissue, as its petrified appearance suggests, but is continuously remodeled through a process of destruction-reconstruction of microscopic portions of its mineralized matrix. This apparently useless process has a great physiological impact: it not only permits the reparation of fatigue lesions and the renewal of old structures, in so doing also leading to new microarchitectural organization if requested by modified mechanical forces, but above all contributes to the regulation of the calcium homeostasis through the release of calcium ions from the calcified matrix. The main control factors of this process are, therefore, the type and strength of the skeletal mechanical activities ('mechanostat') and the calcium ion concentration in the blood. These factors, together or separately, induce the bone cells themselves or extra-osseous cells to produce substances which in turn regulate the formation and the activity of the so-called Basic Multicellular Units (BMUs), i.e., the local responsible for bone remodeling. The BMUs are temporary multicellular complexes that develop on the bone surfaces and run through different functional phases, the first one being the activation of a variable number of osteoclasts (phase of activation). These cells accumulate on the bone surface and reabsorb it to a various extent (phase of resorption) to be replaced, at the end of their activity, by macrophagic cells, which synthesize the so-called cementing zone (phase of reversion). These post-osteoclastic cells are then replaced by active osteoblasts, which synthesize as much bone matrix as it had been reabsorbed by the osteoclasts (phase of ossification).

Each of these phases has its own regulatory mechanism. The activation and resorption phases are mainly regulated by the parathyroid hormone (PTH) and the vitamin D metabolite 1,25(OH)₂D₃, which have a stimulatory activity, and by the calcitonin, which is inhibitory. The development and activation of the osteoclasts are also under the control of local growth factors (TGF-β, MCF, BMP, FGF, IGF) and cytokines (IL-1, IL-6). Moreover, they depend on the concentration of so-called osteoprotegerin (OPG), a member of the tumor necrosis factor receptor family that behaves as a decoy receptor of RANKL. The link of OPG with RANKL prevents the latter for activating RANK, a receptor of hemopoietic cells whose activation in the presence of the M-CSF promotes osteoclast differentiation. Little is known about the regulation of the reversal phase. It has been suggested that growth factors, like for instance BMPs, can be released and activated by the osteoclastic disintegration of the calcified matrix in which they had been embedded during bone formation. Several factors are known to regulate the phase of ossification. Some of them are general factors, like for instance the estrogens, which stimulate osteoblast activity, or the glucocorticoids, which are inhibitors; others are local factors. Among the latter, the canonical Wnt/β-catenin pathway is of particular interest, because it increases the bone mass by stimulating the stem cell formation, the pre-osteoblasts replication and the osteoblast differentiation. The binding of Wnt signaling glycoproteins to the receptors Frizzled family proteins (Fr) and to the low-density lipoprotein receptor-related protein 5 (LRP5) inhibits the phosphorylation of β-catenin by glycogen synthase kinase 3β (GSK-3β), so that β-catenin is not degraded and can accumulate in the nucleus where it activates specific transcription factors for osteoblasts. The process is controlled by antagonists, chiefly by the secreted frizzled-related proteins (sFRPs), which compete with Fz receptors, and by DKK1, a member of the Dickkopf family, which binds to LRP5. Another Wnt antagonist that binds to LRP5 is sclerostin (Sost), which is a product of the old osteocytes; its production is reduced by loading and increased by unloading, so that it represents a possible mechanotransduction system that permits the osteocyte, the mechanosensory cell of the skeleton, to regulate the bone mass. Also regulated by skeletal loads is the osteocyte synthesis of prostaglandin E₂ (PGE₂) that activates the protein kinase A (PKA) that, in turn, induces the stabilization of β-catenin.

The numerous cell factors that control the bone remodeling are integral components of a complex, highly regulated system which permits the resorption phase to be coupled with the ossification phase. Obviously, an uncoupling due to an imbalance of these phases inevitably leads to serious skeletal diseases (osteosclerosis, osteoporosis).

Speaker Abstracts

So2: Animal models for osteoporosis – bone histomorphometric approach

Paola Ballanti

Department of Radiological, Oncological and Pathological Sciences
Sapienza University of Rome, Italy

Osteoporosis is the most common metabolic bone disease characterized by bone mass reduction and altered trabecular microarchitecture that lead to bone fragility and fractures. Such condition is classified as primary or secondary: the primary disease includes idiopathic juvenile osteoporosis and involutional osteoporosis (postmenopausal and senile), while secondary osteoporosis is usually associated with a recognizable disease or medical therapy (e.g. glucocorticoid administration). As well as every metabolic bone disease, the different types of osteoporosis are due by specified alterations of the remodeling process carried out at discrete locations by sequentially coupled teams of osteoclasts and osteoblasts, referred to as the Basic Multicellular Unit. In the last years, much progress has been done in understanding the pathophysiological aspects and improving the therapeutical approaches of the various types of osteoporosis. In this regard, bone histomorphometry, that consists of measuring bone tissue components by microscopic examination of histological sections, has played a fundamental role by providing quantitative information on bone tissue pathological alterations, in comparison with normal condition, and changes induced by therapeutic agents. This technique accurately evaluates bone mass and microarchitecture at a very high resolution, compared with other imaging techniques. It gives the exact amount of the osteoblastic formation activity and the osteoclastic erosion process. After administration of fluorochrome labeling agents (i.e. tetracycline), that are administered at timed intervals and integrate into forming bone, it allows a dynamic analysis of bone remodeling. Standardized nomenclature, symbols, and measurement units of the histomorphometric variables are widely used (1). Such technique was mainly developed after the introduction of plastic-embedded histological sections of undecalcified bone, and of outpatient iliac crest biopsy, by which a sample of both trabecular and cortical bone can be obtained in humans. However, since bone biopsy is generally performed when the disease is in an advanced stage, the disturbances of bone remodeling that led to the reduction in bone mass took place even several years before the time of the biopsy and are no longer evident. Moreover, ethical limits do not allow to test new potential therapies in humans. As a result, a great amount of information has been given by experimental studies in which animal models for human osteoporosis have been used.

Postmenopausal osteoporosis is by far the most common type of osteoporosis, thus most experimental studies have been referred to such type of disease. Postmenopausal bone loss experiments have typically been performed in surgically ovariectomized animals. Several animal models, including rodents, dogs, sheeps, and non-human primates, with their specific advantages and limitations, have been proposed to study estrogen-deficient bone loss and to evaluate the potential therapeutic value of specific pharmaceutical agents (2). Among these models, the ovariectomized rat, whose mineral metabolism and skeletal remodeling cycle have been extensively studied, is the most used (3). In fact, the rapid decrease of cancellous bone volume, together with increase of skeletal remodeling processes that have been described in these animals after ovariectomy, have been considered similar to those that occur in the development of post-menopausal osteoporosis (4). In rats, different skeletal districts respond quite differently to ovariectomy. The metaphyses of long bones (i.e. distal femur, proximal tibia) lose bone more rapidly than other sites of cancellous or cortical bone. Histomorphometrically, the dramatic metaphyseal Bone Volume loss depends on Trabecular Number reduction and Trabecular Separation increase, rather than Trabecular Thickness decrease. Such bone loss predominantly involves the bone trabeculae centrally located within the metaphysis and in direct contact with the diaphyseal marrow circulation, so that these trabeculae appear especially sensitive to estrogen withdrawal. The rapid disappearance of trabeculae, not accompanied by a proportional decrease of trabecular thickness, occurs by intense erosion processes, as demonstrated by the dramatic increase of Osteoclast Surface and Osteoclast Number. Moreover, by considering the specific location within the metaphysis in which trabecular removal occurs, resorption processes appear quite selective, showing a decreasing gradient of intensity from the medullary canal to the cartilaginous growth plate. The histodynamic variables of bone formation, such as Mineral Apposition Rate, are also increased after ovariectomy, due to increased bone turnover. However the bone formation process does not help to prevent the bone loss (5).

Speaker Abstracts

Most early studies on rats lead to the erroneous idea that the rat skeleton was continuously growing throughout life and that rats could not be a good model of human senile osteoporosis. However, several studies have indicated that this is not so (6). Another common criticism was that rats do not normally experience a progressive age-related decline in bone mass that is analogous to the bone loss that occurs with aging in humans. However, rats experience bone loss with advancing age (4). This notwithstanding, while the ovariectomized rat successfully models accelerated bone loss due to menopause, there are, as yet, no generally accepted models for age-related bone loss that normally occurs with aging in all individuals, and predisposes to senile osteoporosis. The species that are being explored to complement the rat model include mice, dogs, sheep, minipigs, ferrets, and nonhuman primates (4). Recently, the transgenic mice model has emerged as a powerful tool to investigate skeletal pathophysiology in molecular and genetic terms. Due to the technology of gene targeting, some surprisingly mild phenotypes in mice with ablation of genes thought to play key roles in bone metabolism have been discovered, combined with numerous genes that previously were not known to impact the skeleton (7). In respect to this, homozygous mutant mice, which lack expression of the *klotho* gene exhibit rapid onset of aging-related disorders including osteopenia, especially in the cortex bones, just like human senile osteoporosis. Histomorphometric variables of both bone formation, e.g. Osteoblast Surface and Bone Formation Rate, and resorption, e.g. Osteoclast Number and Eroded Surface, are lower in *KL*^{-/-} mice than in the wild-type littermates, with predominant decreases of the formation indices, indicating a state of low bone turnover osteopenia. Because this state represents a characteristic feature of senile osteoporosis in humans, *KL*^{-/-} mice can be regarded as a useful model for investigating cellular and molecular mechanisms of age-related bone loss (8).

Glucocorticoid-induced osteoporosis is third in frequency after postmenopausal and senile osteoporosis. Glucocorticoids have both systemic and direct skeletal effects that predispose treated patients to osteoporosis. Since they are used for an extraordinarily large number of disorders, patients' heterogeneity, osseous effects of the underlying diseases, the nonuniformity of dose and duration of therapy might confound the clinical study of glucocorticoid effects on bone in humans. Thus, our understanding of the mechanism(s) of glucocorticoid-induced bone loss in humans has been facilitated by studying the osseous effects of glucocorticoids in animal models. Many studies on corticosteroid effects on bone are performed in growing rats by examining lumbar vertebrae and/or proximal tibia trabecular bone. However, in the proximal tibia, a skeletal site with rapid longitudinal bone growth, corticosteroid excess increases cancellous bone mass. Such finding in the growing rats is explained by the fact that, due to a glucocorticoid induced arrest of the secondary spongiosa remodeling, the calcified cartilage spicules that form a template for new cancellous bone formation are not resorbed, as it occurs in normal condition, and tend to accumulate resulting in an increase in cancellous bone volume. In the slowly growing lumbar vertebra, glucocorticoid administration to young growing rats does not increase cancellous bone mass but does not cause osteopenia. These artifacts of longitudinal bone growth are not expected to occur in adult or aged rats (9). Unlike the rat, it has been reported that the mouse is a faithful animal model of the glucocorticoid-induced bone loss in humans: only in the mouse glucocorticoid administration consistently induces axial greater than appendicular bone loss thus reproducing the major features of the human disease (10). Histomorphometric studies of animal models consistently demonstrate a marked reduction in indices of bone formation, such as reduced Osteoblast Surface and Mineral Apposition Rate, and prolonged Mineralization Lag Time. As a consequence, Wall Thickness, the amount of bone that is replaced in each remodeling cycle, is severely reduced. This process leads to a dramatic and progressive reduction of Trabecular Thickness and loss of cancellous Bone Volume. Some animal studies have shown that during the early phase of glucocorticoid use, the reduced bone formation is associated with a transient increase in Osteoclast Number and Erosion Surface. With continued use of glucocorticoids the rapid rate of bone loss slows down but bone resorption continues to exceed bone formation in relative terms. Bone formation, that remains the most significant event leading to glucocorticoid-induced bone loss, is inhibited through a decrease in osteoblast function and life span (11). The first *in vivo* evidence of the direct action of glucocorticoids on osteoblasts has been determined in transgenic mice overexpressing, specifically in osteocalcin-expressing cells (i.e. osteoblasts), 11 β -hydroxysteroid dehydrogenase type 2, an enzyme that converts biologically active glucocorticoids to inert metabolites. Mice harboring the transgene are protected from glucocorticoid-induced apoptosis of osteoblasts, which in turn results in preventing the decrease in bone formation histomorphometric indices and bone mass (12).

In conclusion, animal models have allowed a remarkable progress in the knowledge of osteoporosis, and need to be applied in further studies to investigate the still unexplored pathogenetic mechanisms and the skeletal safety and activity of new anti-resorptive and anabolic pharmaceutical agents. In the animal model bone research field, bone histomorphometry involving static and dynamic measurements of bone tissue has emerged as the gold standard (4).

Speaker Abstracts

References

1. Parfitt A.M. et Al., *J. Bone Miner. Res.* 2, 595-610, 1987.
2. Rodgers J.B. et Al., *Bone* 14, 369-77, 1993.
3. Lelovas P.P. et Al., *Comp. Med.* 58, 424-30, 2008.
4. Kalu D.N., In: *The Aging Skeleton* (C.J. Rosen et Al. Eds.), pp. 37-50. Academic Press, New York, 1999.
5. Bonucci E. et Al., *Calcif. Tissue Int.* 56, 274-9, 1995.
6. Frost H.M. and Jee W.S.S., *Bone Miner.* 18, 227-36, 1992.
7. McCauley L.K., *Curr. Opin. Rheumatol.* 13, 316-25, 2001.
8. Kawaguchi H., *Rev. Endocr. Metab. Disord.* 7, 17-22, 2006.
9. Li M. et Al., *Bone* 19, 81-8, 1996.
10. Manolagas S.C. and Weinstein R.S., *J. Bone Miner. Res.* 14, 1061-6, 1999.
11. Silvestrini G. et Al., *Bone* 26, 33-42, 2000.
12. O'Brien C.A. et Al., *Endocrinology* 145, 1835-41, 2004

Speaker Abstracts

S03: From development to joint disease: animal models of osteoarthritis

Rik J. Lories

Skeletal Biology and Engineering Centre, Department of Development and Regeneration, KU Leuven, Belgium

Osteoarthritis is the most common chronic joint disorder affecting millions of people worldwide. Its prevalence rises with aging and is reaching epidemic dimensions as risk factors such as obesity continue to increase. In contrast to other joint diseases such as rheumatoid arthritis and spondyloarthritis, no effective treatment for osteoarthritis is currently available. Patients are using painkillers, anti-inflammatory drugs and nutraceuticals and in severe or advanced cases surgical intervention is necessary.

All tissues of the joint are involved in osteoarthritis with progressive cartilage damage, subchondral bone remodeling, osteophyte formation at the joint margins and variable degrees of synovial inflammation. Further progress in the development of adequate interventions is expected from better understanding of the genetics and the molecular mechanisms of disease. Systematic candidate gene approaches and the introduction of the novel genome wide association studies has identified links between osteoarthritis susceptibility and genes known for their roles in bone and joint development. Among these, growth and differentiation factor 5 and frizzled related protein, have been most extensively studied. The generation of transgenic animals with gain or loss of function in genes of interest is an important tool to understand their role in joint biology and disease. A lot of information has become available thanks to the specific development of a number of induced osteoarthritis models. In these setups chemical, surgical or enzymatic approaches are used to mimic damage to the cartilage and to evaluate the resulting progression of damage in the challenged joint. Such approaches appear essential as preclinical modes not only to provide better insights into the data provided by the genetic community but also to bring the identification of pathways towards therapeutic options.

Current research efforts are focusing on a novel gene cluster located on chromosome 7q22 in which different candidate genes are found. Novel animal models for these genes are under development and should provide novel insights into osteoarthritis.

Speaker Abstracts

So4: Mouse models of bone metastasis: pathogenesis and treatment

Thomas J. Rosol, Jessica K. Simmons, Chelsea K. Martin, Jillian L Werbeck, Blake Eason Hildreth III, Gwendolyn Lorch, and Ramiro E. Toribio

Department of Veterinary Biosciences, Ohio State University, Columbus, Ohio, 43210 USA

Bone metastasis is a devastating and frequent outcome for multiple human cancers and is most common as a late-stage manifestation of breast and prostate cancer. Other solid cancers that metastasize to bone include lung, kidney, and thyroid cancer and malignant melanoma. Spontaneous bone metastasis is less common in animal cancers but occurs in dogs and cats with mammary, prostate, bladder, and lung cancer. In addition, oral squamous cell carcinoma often invades bones of the head in humans and cats.

The reasons for cancer metastasis to bone are still poorly understood; however, Sir James Paget (British surgeon and pathologist; 1814-1899) proposed the 'seed and soil' hypothesis, where some tumor cells (seeds) prefer to colonize and grow in bone (soil). This hypothesis is still relevant today, but it is now recognized that there is a dynamic interplay between cancer cells and the bone microenvironment.

Bone-invasive cancers and metastases are incurable. Most cancers, such as breast cancer, typically induce osteolytic metastases. In contrast, prostate cancer induces osteoblastic metastases where there is induction of new bone formation by the prostate cancer cells.

In vivo models of bone metastasis that mimic the human condition are important for understanding the pathogenesis and developing treatments to reduce the incidence and inhibit the growth of bone metastases.

There are well characterized mouse models of bone metastasis for human breast cancer, lung cancer, and malignant melanoma. We modeled osteolytic bone metastases of breast cancer with human MCF-7 cells and mouse PymT cells using intracardiac and intratibial injections. PymT cells metastasized to the ovaries and adrenals, but had few bone metastases after intracardiac injection. The PymT cells formed tumors in bone after intratibial injections that were osteolytic. Estrogen treatment with subcutaneous pellets increased local tumor growth and osteolysis induced by MCF-7 cells after intratibial injection. Osteolytic bone metastases of lung cancer were modeled using intracardiac injections of human HARA squamous carcinoma cells. The HARA bone metastases were very osteolytic and produced high levels of PTHrP with secondary hypercalcemia. PTHrP production and hypercalcemia were dependent on the action of the EGF receptor and were inhibited by gefitinib.

Most mouse models of prostate cancer do not mimic the human condition either because cancer cells do not metastasize to bone or they do not cause osteoblastic metastases. We have developed three cell lines from dogs with spontaneous prostate cancer that mimic human prostate cancer. The canine Ace-1 cells form osteoblastic bone metastases in nude mice after intracardiac injection and do not form metastases in other organs. This model has been effective for studies on prostate cancer pathogenesis and treatment. Stable transfection of the Ace-1 cells with the Wnt inhibitor, DKK1, increased tumor growth in vivo, increased the number of bone metastases, and changed the phenotype of the bone metastases to osteolytic from osteoblastic. Therefore, the Wnt signaling pathway in cancer cells and bone likely plays an important role in the progression of prostate cancer. The canine Leo cells form metastases predominantly in the brain and spinal cord of nude mice after intracardiac injection, but also metastasize to the bones and adrenals. The Leo cells are used to model brain metastasis, since the mice will die from multifocal brain metastases. The canine Probasco cells are a slow growing cell line in vivo that form osteoblastic metastases in mice after intratibial injection. Human prostate cancer is a relatively slow growing cancer with a low proliferation index; therefore, a slow growing tumor may more accurately represent prostate cancer in men.

Bone metastasis is a devastating and frequent outcome for multiple human cancers and is most common as a late-stage manifestation of breast and prostate cancer. Other solid cancers that metastasize to bone include lung, kidney, and thyroid cancer and malignant melanoma. Spontaneous bone metastasis is less common in animal cancers but occurs in dogs and cats with mammary, prostate, bladder, and lung cancer. In addition, oral squamous cell carcinoma often invades bones of the head in humans and cats.

The reasons for cancer metastasis to bone are still poorly understood; however, Sir James Paget (British surgeon and pathologist; 1814-1899) proposed the 'seed and soil' hypothesis, where some tumor cells (seeds) prefer to colonize

Speaker Abstracts

and grow in bone (soil). This hypothesis is still relevant today, but it is now recognized that there is a dynamic interplay between cancer cells and the bone microenvironment.

Bone-invasive cancers and metastases are incurable. Most cancers, such as breast cancer, typically induce osteolytic metastases. In contrast, prostate cancer induces osteoblastic metastases where there is induction of new bone formation by the prostate cancer cells.

In vivo models of bone metastasis that mimic the human condition are important for understanding the pathogenesis and developing treatments to reduce the incidence and inhibit the growth of bone metastases.

We have also developed a mouse model of head and neck squamous cell carcinoma with invasion into the mandible using spontaneous cancers from the oral cavity of cats and humans.

Advanced imaging technologies have facilitated in vivo experiments using the mouse models and include bioluminescent, high resolution radiography, and microCT imaging. Bioluminescent imaging is sensitive enough to visualize a few hundred cells in a bone metastasis in vivo. Bioluminescent imaging of bone metastases in mice enables rapid analysis and quantitation of metastases to measure the effectiveness of drugs in vivo.

It is now recognized that some metastatic cancer cells reside in the bone marrow in a quiescent state (disseminated tumor cells; DTC), even in patients that do not develop metastases. This is an important area of future investigation to understand what is required for the cancer cells to leave dormancy and proliferate. Animal models of dormancy are currently lacking.

Therapy for bone metastases in humans have focused on drugs that inhibit bone resorption and include the bisphosphonates, antibodies and proteins that affect RANK (receptor activator of NF- κ B) ligand signaling in osteoblasts and osteoclasts, and antibodies to bone resorption factors (such as parathyroid hormone-related protein). Inhibition of bone resorption has reduced the number of skeletal related events (SREs) in humans with metastatic cancer; however, it is unknown whether there is an effect on the number or incidence of bone metastases. Other forms of therapy for bone metastases include bone-seeking radioisotopes (⁸⁹Sr and ¹⁵³Sm) and bone modifying agents (endothelin A receptor antagonists, Src inhibitors, Met inhibitors, and cathepsin K inhibitors).

Mouse models of metastasis have proven effective in studying the relationship of cancer cells with the bone marrow microenvironment and investigating novel forms of therapy. It is likely that both the metastatic cancer cells and the bone microenvironment will provide important targets for prevention and therapy of bone metastasis. Studies in our laboratory have shown that the bisphosphonate, zoledronic acid, inhibits bone resorption at sites of metastasis or bone invasion by head and neck cancer. However, bone resorption has not been completely blocked at sites of metastasis, but is effectively blocked in bones without cancer. Therefore, the cancer cells are able to partially overcome the effects of bisphosphonates. In addition, zoledronic acid has not reduced the incidence of bone metastasis in the mouse models using canine prostate and human lung cancer cells.

In summary, bone metastasis is an important and serious outcome for many people with cancer. The pathogenesis of bone metastasis is still poorly understood. The bone microenvironment plays an important role in bone metastasis and the interplay between metastatic cancers and the microenvironment results in modification of cancer cell progression and the pathologic bone phenotype. It is important to prevent bone metastases and limit the growth of metastases once they occur. Drugs that regulate bone resorption or formation directly may help prevent bone metastases or serve as adjuvant palliative forms of therapy.

Speaker Abstracts

References

- Halvorson, KG, Kubota K, Sevcik, MA, Lindsay TH, Sotillo JE, Ghilardi JR, Rosol TJ, Shelton DL, Mantyh PW. A blocking antibody to nerve growth factor attenuates skeletal pain induced by prostate tumor cells growing in bone. *Cancer Research*, 65:9426-9435, 2005
- LeRoy BE, Nadella MVP, Thudi NK, Toribio RE, Tannehill-Gregg SH, Davis D, Corn S, Rosol TJ. New bone formation and osteolysis by a metastatic, highly invasive canine prostate carcinoma xenograft. *The Prostate*, 66:1213-1222, 2006.
- Martin CK, Dirksen WP, Shu ST, Werbeck JL, Thudi NK, Yamaguchi M, Wolfe TD, Heller KN, Rosol TJ. Characterization of Bone Resorption in Novel In Vitro and In Vivo Models of Oral Squamous Cell Carcinoma. *Oral Oncology*, 48 (6): 491-499, 2012.
- Martin CK, Werbeck JL, Thudi NK, Lanigan LG, Wolfe TD, Toribio RE, Rosol TJ. Zoledronic acid reduces bone loss and tumor growth in an orthotopic xenograft model of osteolytic oral squamous cell carcinoma. *Cancer Research*, 70(21): 8607-8616, 2010.
- Park SI, Kim SJ, McCauley LK, Gallick GE. Preclinical mouse models of human prostate cancer and their utility in drug discovery. *Current Protocols in Pharmacology*. Supplement 51: 14.15.1-27, 2010.
- Rosol TJ, Tannehill-Gregg SH, Corn S, Schneider A, McCauley LK. Animal models of bone metastasis. *Cancer Treatment and Research*, 118: 47-81, 2004.
- Schade GR, Keller J, Ives K, Cheng X, Rosol TJ, Keller ET, Roberts WW. Histotripsy focal ablation of implanted prostate tumor in an Ace-1 canine cancer model. *Journal of Urology*, in press, November, 2012.
- Tannehill-Gregg SH, Levine AL, Nadella MVP, Iguchi H, Rosol TJ. The effect of zoledronic acid and osteoprotegerin on growth of human lung cancer in the tibias of nude mice. *Clinical and Experimental Metastasis*, 23(1):19-31, 2006.
- Thudi NK, Shu ST, Martin CK, Lanigan LG, Nadella MVP, Van Bokhoven A, Werbeck JL, Simmons JK, Murahari S, Kisseberth WC, Breen M, Williams C, Chen C-S, McCauley LK, Keller ET, Rosol TJ. Development of a Brain Metastatic Canine Prostate Cancer Cell Line. *The Prostate*, 1251-1263, 2011.
- Thudi NK, Martin CK, Murahari S, Shu SS, Lanigan LG, Werbeck JL, Keller ET, McCauley LK, Pinzone JJ, Rosol TJ. Dickkopf-1 (DKK-1) stimulated prostate cancer growth and metastasis and inhibited bone formation in osteoblastic bone metastasis. *The Prostate*, 71:615-625, 2011.
- Thudi NK, Martin CK, Nadella MVP, Dirksen WP, Werbeck JL, Rosol TJ. Bone Metastasis is Independent of Osteolysis in a Nude Mouse Model of Canine Prostate Cancer with Mixed Bone Metastases. *The Prostate*, 68:1116-1125, 2008.
- Weillbaecher KN, Guise TA, McCauley LK. Cancer to bone: a fatal attraction. *Nature Reviews Cancer*, 11 (6):411-25, 2011.

Speaker Abstracts

S05: In vivo imaging approaches for skeletal muscle & correlations with histopathology endpoints

Kumar Changani
GlaxoSmithkline, Stevenage, UK

Imaging technology provides a powerful means of assessing disease development in the intact, living organism, therefore improving understanding of disease pathology and crucially enabling earlier and more accurate selection of drug candidates. Currently, the major application of imaging technologies to the R&D process is in late preclinical phases through to the clinical phases, but their use in drug discovery is increasing.

Imaging techniques such as MRI, CT, PET and Optical approaches provide high-resolution anatomical, functional, cellular and molecular data that is derived non-invasively. The non invasive nature of imaging is especially important in the study of chronic diseases such as neurodegenerative disorders (Alzheimer's and Parkinson's disease), cardiovascular diseases (atherosclerosis), musculoskeletal disorders, etc. Non invasive imaging studies allow efficacy of drug candidates to be assessed in subjects over relatively long time periods, enabling anatomical and physiological alterations to be compared with the original pretreatment condition. Additionally, longitudinal studies may be conducted on a single animal or patient, such that each subject can act as its own statistical control. This reduces intra-individual variability, increases the statistical quality of the data and subsequently means that fewer animals and patients are required to achieve statistically significant data.

Imaging techniques are increasingly being employed to gain information on pathological changes associated with muscle degeneration. Parameters such as simple volume measurements of specific muscle types can be made to determine the relative efficacy over time of a particular therapy. Importantly, age is a key consideration when determining the relative efficacy of new therapeutics in pre-clinical species and data from MRI measurements of calf muscle will be presented illustrating this fact. Additional non invasive parameters measured by spectroscopic techniques allow determination of the bioenergetic properties of muscle by directly measuring high energy phosphates (nucleotide di and triphosphates) as well as phosphocreatine, inorganic phosphate and glycolytic intermediates such as glucose-6-phosphate and 3-phosphoglycerate. These spectroscopic techniques can be expanded to other important metabolites such as lactate, glucose, glycogen and intra/extramyocellular lipid.

Imaging offers a truly translational approach for assessing disease, as the measurements, analysis and equipment are exactly the same. Understanding clinical disease manifestation through imaging biomarkers helps when designing the most appropriate preclinical species assessment and ultimately serves to improve our confidence in a therapeutic strategy.

Speaker Abstracts

Satellite Session: The Beagle dog brain atlas project

Xavier Palazzi

Sanofi, 13, Quai Jules Guesde, 94400 Vitry sur Seine, France

Beagle dogs are commonly used in preclinical research and neuroscience as the preferred canine model for safety studies. The central nervous system is often a target for pharmaceuticals, medical devices, and chemicals, and a deep knowledge of the gross and microscopic anatomy of the beagle brain is mandatory for anatomic pathologists and neuroscience researchers in order to assess the safety of innovative medicinal and industrial chemicals, or to investigate new mechanisms of action.

Very little literature regarding the anatomy of the beagle brain was available to the scientific community in 2008, as most books were out of print or incomplete, and web-based resources only scant. Hence the decision to build an up-to-date reference atlas in this breed with modern tools and illustrations.

The Beagle Brain in Stereotaxic Coordinates was written for neuroscientists, neuroanatomists, neuropathologists, neurosurgeons as well as students, and it provides a comprehensive guide for those – both researchers and undergraduates – who are interested in the dog brain and comparative anatomy. The nomenclature and atlas presentation were inspired from existing reference brain atlases in the dog and in other species (see references below).

This presentation will detail the history and methodology of realization of the atlas, and will show through practical examples based on the functional anatomy of the motor pathways, how to use the atlas as a reference document to localize the areas of interest.

References

- Adrianov O.S., Domino K.E., Domino E.F. (2010). Atlas of the Canine Brain. Npp Books
Eidelberg E., Saldias CA. (1960) A stereotaxic atlas for Cebus monkeys. J Comp neurol. 115:103-23
Lim R.K.S, Liu CN, Moffitt R.L. (1960). A stereotaxic atlas of the dog's brain. Thomas.
Mai JK., Asshewer J., Paxinos G. (2004). Atlas of the human brain. 2nd ed. Academic Press.
Palazzi X., Bordier N. (2008). The marmoset brain in stereotaxic coordinates. Springer
Paxinos G. (1995). The rat nervous system. 2nd ed. Academic Press.
Paxinos G., Watson C. (2007). The rat brain in stereotaxic coordinates. 6th ed. Academic Press.
Paxinos G., Huang XF. (2009). The Rhesus monkey brain in stereotaxic coordinates. 2nd ed Academic Press.
Saavedra JP., Mazzuchelli AL. (1968). A stereotaxic atlas of the brain of the marmoset (hapale jacchus). J. Hinforsch, 11(1):105-22
Singer, M. (1962). The Brain of the Dog in Section. Saunders.
Stephan H., Baron G., Schwerdtfeger WK. (1980). The brain of the common marmoset (Callithrix jacchus): a stereotaxic atlas. Springer-Verlag.

Speaker Abstracts

So6: Bone imaging – from bench to bedside

Michaela Kneissel

Novartis Institutes for Biomedical Research, Basel, Switzerland

Non-invasive quantitative X-ray based methods such as Dual Energy X-ray Absorptiometry (DEXA) and peripheral quantitative and micro Computed Tomography (pQCT, microCT) allow for evaluation of bone mineral mass, density, geometry and structure in preclinical rodent and nonhuman primate models of bone disorders such as osteoporosis or abnormal bone overgrowth. These imaging techniques are fully translational and have been either extensively used since decades (DEXA) or are increasingly applied (CT) in the clinic. They are utilized in basic research and drug discovery likewise as in clinical settings for disease diagnosis and for monitoring of the efficacy of bone active drugs. Examples will be provided which exemplify the translational nature of these imaging techniques and the predictability of results obtained in preclinical animal models for the human situation (e.g. drug discovery and development for anti-resorptive and bone forming osteoporosis drugs). Finally a brief overview will be given on non-invasive imaging techniques, which allow detecting changes in local bone formation and resorption in preclinical setting. Although some of these techniques may never become fully translational, they allow for a faster and more dynamic evaluation of changes in bone turnover than conventional invasive bone histomorphometric evaluations, which can be only performed ex vivo in smaller species or require a bone biopsy in larger species including humans.

Speaker Abstracts

S07: Clinical pathology & biomarkers for bone & muscle in preclinical safety studies

David Ledieu

Novartis Institutes for Biomedical Research, CH-4002 Basel, Switzerland

The musculoskeletal system can be a target for drug-induced injuries and in the last few years, drug-induced musculoskeletal injuries have become increasingly more recognized, especially due to statins (myalgia and myopathy/rhabdomyolysis), fluoroquinolones (arthropathies and tendinopathies), corticosteroids (tendinopathies, myopathies, and osteoporosis), bisphosphonates (osteonecrosis), and retinoids (hyperostosis, premature epiphyseal closure).

In parallel, the incidence of musculoskeletal injuries is increasingly more prevalent in preclinical evaluation of new therapeutic targets. Several blood (plasma/serum) biomarkers commonly used in preclinical safety studies can provide useful information on the musculoskeletal system. Creatine kinase and lactate dehydrogenase are sensitive biomarkers of skeletal muscle injury but are also present in cardiac muscle and thus cardiac toxicity/myocardial injury must be excluded in order to use them effectively as biomarkers. Alanine aminotransferase and aspartate aminotransferase are also sensitive biomarkers of skeletal muscle injury; however, these lack tissue specificity as well. On the other hand, changes in alkaline phosphatase, calcium, and inorganic phosphate/phosphorus may reflect altered bone metabolism. However, all these biomarkers lack sensitivity and specificity and consequently preclinical detection of musculoskeletal toxicity is typically confirmed by histopathologic evaluation, with standard clinical pathology parameters serving only as adjuncts to the histopathologic findings. Nevertheless, in human medicine and clinical trials, creatine kinase still remains the standard biomarker to define drug induced myopathy. Building on the need for more performant biomarkers of musculoskeletal injuries, additional biomarkers as been proposed to assess skeletal muscle toxicity (e.g., skeletal muscle troponin, parvalbumin, fatty acid-binding protein), bone formation (e.g., bone-specific alkaline phosphatase, osteocalcin, collagen propeptides), and bone remodeling (e.g., osteoprotegerin, osteoprotegerin ligand, collagen telopeptides, pyridinolines).

These non-standard biomarkers may be useful to investigate, monitor, and/or predict musculoskeletal toxicity in preclinical safety studies. Numerous kits have been developed for plasma/serum or urine biomarkers and are now commercially available. Some multiplexed kits are also commercially available enabling to measure multiple biomarkers simultaneously. Optimal use and interpretation of these biomarkers require a good understanding of biological variability, potential preanalytical errors arising from problems with sample collection and processing, and analytical performance limits.

Speaker Abstracts

So8: Comparative long-term preclinical safety evaluation of two glatiramoid compounds in rats and monkeys, with particular emphasize on the subcutaneous toxicity and related systemic changes

Abraham Nyska

Sackler School of Medicine, Tel Aviv University, Israel and RTC Spa, Rome, Italy

Glatiramer acetate (GA) is widely used for the treatment of multiple sclerosis, and was shown to have an excellent safety profile. Glatiramoids are complex polypeptide mixtures that share the same structural formula of GA, but differ in the amino acid sequence. Recently, a new glatiramoid with a higher molecular mass, protiramer, was developed. Our aim is to describe the findings in the long-term toxicity studies with protiramer in rats and monkeys, in comparison to similar studies with GA. The toxicity of subcutaneous GA (0-30 mg/kg/d) or protiramer (0-60 mg/kg in monkeys and 0-300 mg/kg in rats, twice weekly) was evaluated for 26 weeks in SD rats and for 52 weeks in cynomolgus monkeys. While in the GA studies no treatment-related mortalities were noted, protiramer administration led to several mortalities in rats and monkeys. Protiramer administration resulted in severe dose-dependent injection-site lesions, including necrosis, cavity formation, and inflammation, bridging fibrosis in the liver and severe progressive nephropathy in rats. The cause of death was complications related to injection site necrosis. Additionally, a dose-related increase in eosinophil counts was observed in the monkey study. Contrastively, although GA was administered more frequently, injection-site lesions were usually mild and well-tolerated, without systemic effects. The protiramer toxicity studies show that minor variations in the manufacturing of glatiramoids may result in an altered safety profile, and lead to significant toxic effects. It is essential that any new glatiramoid will be carefully studied in long-term preclinical animal studies to ascertain its safety.

Speaker Abstracts

S09: Review of rodent bone tumors induced by parathyroid hormone and related peptides

John L. Vahle,
Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, USA

Parathyroid hormone (PTH) has long been known as a key hormone in bone and mineral ion metabolism. Veterinarians and physicians classically associated increased parathyroid hormone concentrations with detrimental effects on bone based on the metabolic bone disease observed in certain forms of hyperparathyroidism. However, as early as the 1920's the ability of extracts of the parathyroid gland to stimulate bone formation in rodents was established. Beginning in the 1970's there was renewed interest in the positive effects of parathyroid hormone on bone mass and investigators established in that single daily injections of parathyroid hormone and various related peptide fragments can increase bone mass across multiple mammalian species. This body of work eventually led to the formal drug development programs with extensive preclinical and clinical studies characterizing the safety and efficacy of intermittently administered parathyroid hormone.

While animal studies play an important role in all drug development programs, findings from animal studies were particularly pivotal in the course of the development of parathyroid hormone. This presentation will review key animal pharmacology and toxicity studies conducted with parathyroid hormone. Particular emphasis will be placed on the finding of bone neoplastic lesions in the 2-year rat carcinogenicity assays. In these near-lifetime rodent assays, parathyroid hormone treatment resulted in a spectrum of bone proliferative lesions, including osteosarcoma. The neoplastic lesions were observed in the setting of marked increases in bone mass and alterations in bone geometry. In addition to describing the original rodent findings, the presentation will review studies in both rats and monkeys which better defined the effects of dose, treatment duration, and age at initiation on the development of the bone tumors. Quantitative analyses such as densitometry, histomorphometry, and biomechanics were key endpoints in understanding the differences in species to the anabolic effects of teriparatide and the use of these techniques in the animal safety studies will be described. Finally, the impact of the rodent tumor studies on the drug development process and product labeling be reviewed.

References

- Tashjian, A.H. and Goltzman, D. (2008). On the Interpretation of Rat Carcinogenicity Studies for Human PTH(1-34) and Human PTH(1-84). *J Bone Miner Res* 23, 803-811.
- Vahle, J.L., Sato, M., Long, G.G., Young, J.K., Francis, P.C., Engelhardt, J.A., Westmore, M.S., Linda, Y., and Nold, J.B. (2002). Skeletal changes in rats given daily subcutaneous injections of recombinant human parathyroid hormone (1-34) for 2 years and relevance to human safety. *Toxicol. Pathol.* 30, 312-321.
- Vahle, J.L., Zuehlke, U., Schmidt, A., Westmore, M., Chen, P., and Sato, M. (2008). Lack of bone neoplasms and persistence of bone efficacy in cynomolgus macaques after long-term treatment with teriparatide [rhPTH(1-34)]. *J. Bone Miner. Res.* 23, 2033-2039.

Speaker Abstracts

S10: Brown adipose tissue and effects on the morphology

Elke Atzpodien, Rachel Neff, Thomas Singer, Mudher Albassam, Barbara Lenz
Non-Clinical Safety, F. Hoffmann-La Roche Ltd., Basel, Switzerland

Mammals including humans have three main adipose tissue depots: visceral white adipose tissue, subcutaneous white adipose tissue (WAT) and brown adipose tissue (BAT). Visceral WAT is associated with an increased risk of insulin resistance, metabolic syndrome, type 2 diabetes and mortality. Subcutaneous WAT and BAT are insulin sensitive and benefit metabolism by improving glucose homeostasis and increasing energy consumption (Tran TT & Kahn CR, 2010).

The primary function of BAT is in the generation of heat. Reflecting their functional role, brown adipocytes have a large volume of cytoplasm that contains multiple small lipid droplets stored in a multilocular pattern and abundant mitochondria which give their brown coloration. In addition, BAT is highly vascularized and innervated with noradrenergic fibers (Ravussin E and Galgani JE, 2011). Mitochondria are central to BAT activity, releasing chemical energy in the form of heat by means of uncoupling of oxidative phosphorylation. This phenomenon of BAT thermogenesis is mediated by uncoupling protein 1 (UCP1) within the inner mitochondrial membrane. UCP1 renders fatty acid oxidation inefficient, leading to energy dissipation, release of energy as heat, rather than storing it as adenosine triphosphate (ATP). BAT was originally observed in hibernators and was early referred to as “the hibernation gland” (Cannon B and Nedergaard D, 2004).

In mammals, BAT is found in anatomically discrete depots, predominantly the interscapular, periaortic and axillary regions. As BAT plays an important role in the maintenance of body temperature, until recently, its presence was thought to be relevant only in small mammals and infants, with little physiologic relevance in adult humans (Cannon B and Nedergaard D, 2004). However, studies using ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG) positron emission tomography-computed tomography (PET-CT) in humans prove that BAT is present in adults, located mainly in the cervical, supraclavicular, axillary and paravertebral regions, with a higher prevalence in women and leaner individuals. BAT activity is increased in cold environment and reduced with increasing obesity (Cypess AM et al., 2009; Ravussin E and Galgani JE, 2011; Tam CS et al., 2012, van Marken Lichtenbelt WD et al., 2009; Virtanen KA et al., 2009).

A range of hormones including catecholamines, prostaglandins, thyroid and sex hormones, affect brown adipocyte activity, and sympathetic stimulation via the β_3 -adrenergic receptor enhances thermogenesis (Stephens M et al., 2011).

Speaker Abstracts

S11: Preclinical models for devices/biomaterials for the skeletal system and examples of clinical translation

Fini Milena ^{1,2}, Nicoli Aldini Nicolò ^{1,2}, Sartori Maria ²

¹Laboratory of Preclinical and Surgical Studies, Rizzoli Orthopedic Institute, Bologna-Italy

²Laboratory of Biocompatibility, Technological Innovations and Advanced Therapies, Rizzoli RIT Department, Rizzoli Orthopedic Institute

Preclinical investigations are required to assess biomaterials to evaluate toxic, immunogenic, inflammatory response, carcinogenic, mutagenic or teratogenic effects and can be defined as *laboratory or animal studies that are performed to provide evidence that a new treatment is safe and effective enough to be administered in humans*. However, biological preclinical studies are only one step in the most complex and articulated route to validate a material proposed for clinical use; indeed the planning of a research project is based on the contributions of many skills, and the reliability of the results is the direct effect of the appropriate development of the experimental design.

For the preclinical evaluation of biomaterials and devices, over the past decades a wide ranging and comprehensive set of *in vitro* and *in vivo* tests have been developed. By following the regulatory authorities nowadays both *in vitro* and *in vivo* investigations are required because it is thought that the ability of a material to perform a specific function cannot be evaluated only *in vitro* or *in vivo* and that the *in vitro* tests used up to now do not exclude and replace the *in vivo* tests, even if the development and evaluation of new or modified *in vitro* systems are under study (1). According to regulations and guidelines, the *in vivo* tests are justified only when the expected results are not otherwise available and are essential for the material characterization, when no other scientifically validated method not involving animals is proposable and after the acquisition of data obtained by *in vitro* evaluation. The strategies to minimize pain, suffering, distress and lasting harm must be carefully considered, as the animal welfare, anesthesia and procedures of euthanasia.

The limits of both *in vitro* and *in vivo* methods for the evaluation of biomaterials in the skeletal system derive from the complexity of bone tissue which can be only partially experimentally reproduced. Skeletal texture is the combination of many cell types and components: bone, cartilage, connective, mesenchymal, vascular and hemopoietic tissues. Due to differences in architecture, vascularization, and mechanical stress, the healing capacity of the site after implantation greatly affects both osteointegration and biodegradation of biomaterials or devices. Osteointegration is usually greater in cortical bone than in trabecular bone or the medullar region while the degradation rate is greater in medullary and trabecular sites than in cortical bone. From a mechanical point of view, the forces acting on the implants in the tibia or femoral diaphysis are mainly shear forces, whereas the forces acting on the implants in the femoral condyle or tibial plateau are a combination of shear and compression forces. The human skeleton remodels much more than that of other mammals and the bone remodeling around an implant is a long lasting process. Nowadays the evaluation of biocompatibility cannot ignore the development of very complex methods including the use of co-cultured cells and bioreactors that can better mimic some of the complexity of the whole organism. While it is known that tissue healing involves the response of different kinds of resident or migratory cells, *in vitro* biocompatibility is quite often performed by a large battery of *in vitro* tests with different cell types other than in the contemporary presence of these and despite abundant evidence that 2D and 3D models are superior to monolayer cultured cells, the majority of preclinical current research on orthopedic biomaterial biocompatibility relies heavily on the last methods. Co-cultures are attractive also for *in vitro* biomaterial testing because they are one step closer to natural conditions and allow some aspects of the complex interactions among different cell types living in the same microenvironment to be studied, such as between bone-building and bone-resorbing cells, mesenchymal stem cells and differentiated cells, osteoblasts and chondrocytes, chondrocytes and ligamentocytes, fibroblasts and macrophages.

The development of 3-D and multicellular *in vitro* models for biocompatibility testing will also reduce the number of animal experiments. From this point of view, despite vast cultural and economic support for the development of *in vitro* tests on biomaterial safety and biocompatibility these may be improved and validated because there is still the lack of actual alternative methods. This is an important aspect to be supported.

In vivo, biomaterial osteoconductivity, osteoinductivity and osteointegration are studied by heterotopic (subcutaneous and intramuscular tissues) and orthotopic implantation in bone (usually the diaphysis and the epiphysis or both).

Speaker Abstracts

Femur and tibia of medium and large size animals are the long bone segments usually selected for the implants (2). To evaluate osteointegration (the direct structural and functional connection between living bone and the surface of the implant) histomorphometric measures can be obtained by image analysis systems. The most common parameters considered for osteointegration are “Affinity Index” (the length of bone directly opposed to the implant/total length of bone implant interface x 100%) and “Bone ingrowth” (the area of the bone tissue that has grown inside the screw thread/area inside the screw thread) (3). When the implant is placed in the cancellous bone, the quality of the tissue can be also evaluated according to Parfitt formulas (4). The rate of bone deposition and mineralization are evaluated by dynamic histomorphometry. Mechanical tests are also necessary to evaluate the integration of the material with the bone and the bone quality (5, 6).

To date, biocompatibility and biological biomaterial evaluations often fail to take into account that host variables are as important as biomaterial/device variables (7). Therefore, we need testing strategies that take into consideration many other aspects from both the material and the host site. Following ISO rules, sources of cells for cultures usually range from established cell lines to cells isolated from normal and healthy tissues have to be used. Tests on healthy cells and animals, in the preclinical evaluation of biomaterials/devices could not be considered as the final step. In fact, when the biomaterials are implanted into the lesion site the scenario completely differs from the healthy and strictly controlled *in vitro* or *in vivo* conditions. The final product will be implanted often in patients of advanced age, with comorbidities, undergoing chronic drug treatments, or leading unhealthy lifestyles, all of which affect the local microenvironment of the implantation site and bone response. These conditions may affect the behavior and biocompatibility of the biomaterial and the final outcome.

Moreover, the impact of sophisticated processing parameters to develop the correct chemistry and topography of biomaterials in orthopedic surgery requires the use of an advanced methodology for *in vitro* and *in vivo* preclinical testing. For example, nanotechnology is considered to be one of the key technologies of the 21st century but objects on nanoscale differ greatly from bulk materials, as far as biocompatibility and toxicology are concerned. Therefore, standardized, reliable, robust, reproducible and intelligent testing strategies are urgently needed and may be validated to test the biocompatibility of new biomaterials and devices.

Protease, growth factors and pro-inflammatory cytokines are locally produced by cells and the effect of the microenvironment on biomaterials may be marked especially in the first days after surgery. The potentially negative role of the inflammatory environment, because of the lesion or implantation surgery, should be taken into account and appropriate experimental models are therefore required to mimic local and systemic conditions of clinical relevance by changing pH, oxygen tension and, for example, by adding the main cytokines, enzymes and signal molecules involved in healing processes and present in the lesion site.

The use of pathological tissue-derived cells and *in vivo* animal models of diseases should be improved and adopted.

The use of *in vitro* tests with pathological bone-derived cell cultures, such as osteoblasts from the osteoporotic or osteoarthritic bone and cartilage (8,9), in association with *in vivo* disease models, allows the evaluation of the response of bone to candidate orthopaedic biomaterials/devices and especially will permit the clinical translation of experimental results.

By using both *in vitro* and *in vivo* pathological models of human diseases (osteoporosis, osteoarthritis, infection) the clinical translation of devices for joint prostheses, bone implants, large bone defect replacement, osteochondral defects and osteomyelitis treatment will be described.

Speaker Abstracts

References

- 1) Fini M, Giardino R. *In vitro* and *in vivo* tests for the biological evaluation of candidate orthopedic materials: benefits and limits. *Journal of Applied Biomaterials & Biomechanics* 2003;1:155-163
- 2) Giavaresi G, Fini M, Chiesa R, Giordano C, Sandrini E, Bianchi AE, Ceribelli P, Giardino R. A novel multiphase anodic spark deposition coating for the improvement of orthopedic implant osseointegration: an experimental study in cortical bone of sheep. *J Biomed Mater Res A*. 2008; 85(4):1022-31.
- 3) Giavaresi G, Chiesa R, Fini M, Sandrini E. Effect of a multiphasic anodic spark deposition coating on the improvement of implant osseointegration in the osteopenic trabecular bone of sheep. *Int J Oral Maxillofac Implants*. 2008 23(4):659-68.
- 4) Parfitt AM. Bone histomorphometry: proposed system for standardization of nomenclature, symbols, and units. *Calcif Tissue Int*. 1988;42(5):284-6.
- 5) Borsari V, Fini M, Giavaresi G, Tschon M, Chiesa R, Chiusoli L, Salito A, Rimondini L, Giardino R. Comparative *in vivo* evaluation of porous and dense duplex titanium and hydroxyapatite coating with high roughnesses in different implantation environments. *J Biomed Mater Res A*. 2009 ;89(2):550-60.
- 6) Bacchelli B, Giavaresi G, Franchi M, Martini D, De Pasquale V, Trirè A, Fini M, Giardino R, Ruggeri A. Influence of a zirconia sandblasting treated surface on peri-implant bone healing: An experimental study in sheep. *Acta Biomater*. 2009 ;5(6):2246-57.
- 7) DF Williams, "Handbook of Biomaterial Properties, 1998
- 8) Torricelli P, Fini M, Giavaresi G, Giardino R. *In vitro* models to test orthopedic biomaterials in view of their clinical application in osteoporotic bone. *Int J Artif Organs*. 2004;27(8):658-63.
- 9) Torricelli P, Fini M, Giavaresi G, Rimondini L, Tschon M, Rimondini R, Carrassi, A, Giardino R. Chronic alcohol abuse and endosseous implants: linkage of *in vitro* osteoblast dysfunction to titanium osseointegration rate. *Toxicology*. 2008;243(1-2):138-44.

Speaker Abstracts

S12: Preclinical safety evaluation of devices / biomaterials for the musculo-skeletal system

Xavier Palazzi

Sanofi, 13, Quai Jules Guesde, 94400 Vitry sur Seine, France

Following a review of the definition, classification, diversity and importance of medical devices with a special emphasis on devices targeting the musculo-skeletal system, this presentation will briefly review the main regulatory requirements and guidelines to access to the European and American markets and will provide a comprehensive review of the tests required to evaluate the preclinical safety of medical devices in the context of ISO-10993 guidelines.

The specificities of the preclinical evaluation of devices when compared to drugs or biologics will be underlined. A peculiar emphasis will be given on the ISO-10993 part 6 guideline that details implantation studies as one of the means to evaluate biocompatibility. In this context, a general description of special histotechnology for hard tissues and of pathological endpoints and points to consider for the pathologists will be described.

In addition, examples of studies combining both preclinical safety and performance endpoints will be given regarding bone metal implants, polymer matrices, and dental art.

This presentation will be realized with the scientific and iconographic support of NAMSA, France.

Speaker Abstracts

S13: Enabling 3D fabrication technologies to generate cell-instructive porous biomaterials for skeletal regenerative therapies.

Lorenzo Moroni, Wim Hendrikson, Anne Leferink, Andrea Di Luca, Febriyani Damanik, ElaheMasaeli, Anandkumar Nandakumar, Gustavo A. Higuera, and Clemens van Blitterswijk
University of Twente, Tissue Regeneration Department, Enschede, The Netherlands

A key factor in scaffold-based tissue and organ regeneration relies on enhancing cell-material interactions to obtain the same original functionality. Different approaches include delivery of biological factors and surface topography modifications. Although both strategies have proved to augment cell activity on biomaterials, they are still characterized by limited control in space and time, which hampers the proper regeneration of complex tissues. Here, we present a few examples where integration of scaffold fabrication platforms allowed the generation of a new library of scaffolds with tailored physico-chemical cues at the macro, micro, and nano scale. These porous biomaterials sustained the regeneration of both subchondral bone and articular cartilage separately or in an osteochondral construct. From these examples as well as from the study of other scientists, converging technologies seems to be a powerful route towards designing of 3D scaffolds with instructive properties able to control cell activity for the regeneration of functional tissues. Future efforts should aim at further improving technology integration to achieve a fine control on cell fate by scaffold design at multiple scales. This will enable the regeneration of complex tissues including vasculature and innervation, which will result in enhanced *in vivo* integration with surrounding tissues. By doing so, the gap from tissue to organ regeneration will be reduced, bringing regenerative medicine technologies closer to the clinics.

Speaker Abstracts

S14: Histopathological effects of phosphodiesterase type IV (PDE IV) inhibitors on the skeletal system of rodents

Anke Heuser¹, Ursula Junker², Itai Bab³

¹Nycomed: A Takeda Company, Barsbüttel, Germany

²Novartis Pharma AG, Basel, Switzerland

³The Bone Lab, The Hebrew University, Jerusalem, Israel

Bone alterations are observed after treatment of rats with dual selective inhibitors of phosphodiesterase 3/4 (PDE III/IV) as well as with some selective inhibitors of phosphodiesterase 4 (PDE IV). Clinically the effects present as edematous, red and painful swelling of the distal extremities, predominantly of the hind limbs, which often occurs only unilaterally and decreases with time. Radiographically, the bone alterations start as focal new periosteal primary bone formation, which is only minimally mineralized. The new bone keeps accumulating with time, becomes more diffusely mineralized and spreads along the bony shaft. In later stages the new bone develops into mature trabecular bone with a new cortex in its periphery. Histologically, the first alterations are edema and inflammation of the soft tissue around the original bone with fibroplasia, increased vascularization and periosteal thickening. Later stages include the new bone formation around the pre-existing cortical bone as well as hyaline cartilage. The mature trabeculae are oriented perpendicular to the pre-existing cortex. These trabeculae are enveloped by the new cortex with the intertrabecular spaces containing bone marrow tissue with adipose and hematopoietic cells. Inflammation is no longer present.

The alterations are consistent with hypertrophic osteopathy. The condition is also known to occur in other species, including humans and dogs. It occurs often secondary to systemic diseases, intrathoracic neoplasias being the most common. The exact pathogenesis of the disease is however not known. It is suggested that increased blood flow to the distal extremities might be involved. Vascular smooth muscle relaxation after treatment with phosphodiesterase inhibitors may be contributing factors.

In addition, it is reported that phosphodiesterase IV inhibitors can prevent cancellous bone loss in adult ovariectomised mice.

Speaker Abstracts

INHAND 01: Proliferative and non-proliferative lesions of the skeletal system and tooth in rodents

Part 1: Neoplastic lesions

Heinrich Ernst

Fraunhofer Institute of Toxicology and Experimental Medicine (ITEM), Hannover, Germany

Proliferative lesions of the skeletal system and tooth have a very rare spontaneous occurrence in laboratory rodents. The incidence of osteosarcoma in aged rats and mice ranges between 0.1% and 1%, whereas the frequency of all other skeletal system and tooth tumor types in both species is about 10fold lower (0.01% to 0.1%; source: RITA database). Neoplasms of the skeletal system may also be induced by ionizing radiation, viruses, genotoxic chemicals or exogenous hormones such as diethylstilbestrol (DES).

In this interactive session, several of the proliferative lesions listed below will be shown and discussed with consideration of related differential diagnoses. The presented cases are going to be from rat, mouse or Syrian hamster and everybody in the audience will be asked to provide her/his diagnosis.

Proposed Terminology of Proliferative Lesions of the Skeletal System and Tooth in Rats and Mice

I. Bone, Cartilage, Joint

- Hyperplasia, Osteoblast
- Osteoma
- Osteoblastoma
- Osteofibroma (Mouse only)
- Osteosarcoma
- (Primary Bone) Fibrosarcoma
- Hyperplasia, Cartilage
- Chondroma
- Osteochondroma (Rat only)
- Chondrosarcoma
- Chordoma, Benign (Rat only)
- Chordoma, Malignant (Rat only)
- Hyperplasia, Synovial Cell
- Sarcoma, Synovial

II. Tooth

- Ameloblastoma
- Odontoma, Ameloblastic
- [incl. Fibro-Odontoma, Ameloblastic (Mouse only)]
- Odontoma, Compound
- Odontoma, Complex
- Fibroma, Odontogenic
- Fibroma, Ossifying/Cementifying (Mouse only)
- Tumor, Odontogenic, Benign
- Tumor, Odontogenic, Malignant

Speaker Abstracts

Part 2: Non-neoplastic lesions

Matthias Rinke

Bayer Pharma AG, Wuppertal, Germany

Non-proliferative changes of the skeletal system (bone and joints) and teeth may occur as a spontaneous – mostly degenerative – finding, but in most cases, they are related to treatment, especially with compounds that are intended to be used in cancer therapy.

In the second part of this interactive session, several non-proliferative lesions of the skeletal system and from teeth will be shown and discussed with consideration of related differential diagnoses. The presented cases are going to be from rats and mice and everybody in the audience will be asked to provide her/his diagnosis by our voting machines.

The terms presented are in concordance with the proposed terms of the INHAND group. To avoid redundancy, it is here referred to the INHAND Poster Abstracts 02 and 03.

Reference

Leininger J, Bendele A, Bolon B, Boyce R, Ernst H, Fossey S, Healy L, High W, Jolette J, Long P, Ozaki K, Rinke M, Roth D, Schelling S, Vahle J. International Harmonization of Nomenclature and Diagnostic Criteria (INHAND): Proliferative and Non-Proliferative Lesions of the Skeletal System of the Rat and Mouse (Tox. Path., in preparation)

Speaker Abstracts

INHAND 02: INHAND Update – Mammary gland

*Karin Küttler, Heike Antje Marxfeld, Silke Treumann
BASF SE, Ludwigshafen, Germany*

The INHAND Project (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) is a joint initiative of the Societies of Toxicologic Pathology from Europe (ESTP), Great Britain (BSTP), Japan (JSTP) and North America (STP) to develop an internationally-accepted nomenclature for proliferative and non-proliferative lesions in rodents used in regulatory as well as research studies. The working group for the mammary gland was comprised of representatives from the European, British, Japanese, and North American Societies of Toxicologic Pathology. The working group discussed several issues at the process of establishing the new nomenclature and diagnostic criteria. For example, size criteria for dilation (galactoceles) or different mixed tumors of the mammary gland were discussed. Another topic for debate was the differentiation of lobuloalveolar hyperplasia and tumors.

Selected proliferative lesions of the mammary gland will be presented. During the presentation electronic audience voting on the selected cases will be available. Following voting, audience discussion of the case presentations will be encouraged.

The work of the INHAND mammary working group will be published in a Toxicologic Pathology Supplement this year (2012) and is electronically available on the internet (<http://goreni.org>) in a pre-published accepted version.

Case Presentations

Case 1: Francesco Marchesi, Elena Riccardi and Anna Maria Giusti

Species, strain	Sprague-Dawley rats	
Sex	Female	Male
Age	Young adult (12 weeks)	Aged (105 weeks)
Study type	6-week repeat dose toxicity study	2-year carcinogenicity study
Treatment	Test article low dose	Vehicle control
Clinical findings	None	None
Organ(s)	Kidney	Kidney
Gross finding(s)	None	Abnormal yellow colored area, 10 mm in diameter, on the cranial pole of the left kidney
Staining	H.E.	H.E.

Case Presentations

Case 2: Céline Thuilliez

Species, strain	Göttingen minipigs
Sex	Males and females
Age	NA
Study type	NA
Treatment	None
Clinical findings	NA
Organ(s)	Various
Gross finding(s)	NA
Staining	H.E. and/or PAS

Case Presentations

Case 3: Elena Riccardi

Species, strain	Beagle dog
Sex	Male
Age	Adult
Study type	26-week oral toxicity study
Treatment	Control item
Clinical findings	NA
Organ(s)	Adrenal gland
Gross finding(s)	Cyst in the cortex, 1 mm in diameter
Staining	H.E., PAS, Alcian blue, Masson's Trichrome, IHC

Case Presentations

Case 4: Anna Maria Giusti, Elena Riccardi

Species, strain	Beagle dog
Sex	Male
Age	Adult
Study type	Repeated intra-arterial toxicity study
Treatment	NA
Clinical findings	Gingival overgrowth
Organ(s)	Mucosa of the oral cavity
Gross finding(s)	NA
Staining	H.E.

Case Presentations

Case 5: Dirk Nehrbass, Arens, D., Zeiter S.

Species, strain	Rabbit, New Zealand White
Sex	Female
Age	25 weeks
Study type	NA
Treatment	None
Clinical findings	Hard swelling around both knees
Organ(s)	Bone and joint (tibia, femur, patella, knee joint)
Gross finding(s)	Femoro-patellar joint: capsule thickened, rigid consistency, white beige coloured, bilateral Articular fluid: viscous, clear, not cloudy, yellow (bacterial investigation negative) Patella: patellar dislocation proximally to the trochlea ossis femoris, ligamentum patellae thickened, bilateral Muscle quadriceps femori: shortened and reduced in volume (atrophy), bilateral Tibia: tuberositas tibiae radiologically not fully connected to tibial shaft, bilateral Femur: area of proximal of trochlea shows irregular surface lining, bilateral
Staining	Giemsa-Eosin

Case Presentations

Case 6: Enrico Radaelli, Morvarid Farhang Ghahremani, Michele Ardizzone

Species, strain	Mouse, mixed genetic background
Sex	Female
Age	26 weeks
Study type	NA
Treatment	None
Clinical findings	Generalized malaise, irregular bilateral swelling of inguinal and abdominal fat pads
Organ(s)	Complete necropsy
Gross finding(s)	Mild splenomegaly; mild hepatomegaly; marked and diffuse whitish thickening of subcutis/mammary fat pad in the abdominal, inguinal and perineal regions; moderate and locally extensive thickening of subcutis in the dorsal region; diffuse whitish thickening of peritoneal ligaments
Staining	H.E. and IHC

Case Presentations

Case 7: Vittoria Castiglioni, Alessandra Rustighi, Eugenio Scanziani, Enrico Radaelli

Species, strain	Mouse, C57BL/6 genetic background
Sex	Male
Age	15-week-old
Study type	NA
Treatment	None
Clinical findings	Abdominal enlargement, generalized deterioration of clinical conditions
Organ(s)	Complete necropsy
Gross finding(s)	Ascites; left kidney, completely effaced and enlarged (1.5x3 cm in diameter) by a lobulated, tan and hemorrhagic mass
Staining	H.E. and IHC

Case Presentations

Case 8: Anna Lanzoni

Species, strain	Rat
Sex	Males and females
Age	NA
Study type	4-week oral repeat dose toxicity study with a 2 week recovery period
Treatment	NA
Clinical findings	NA
Organ(s)	Femur (femoro-tibial joint) ; sternum
Gross finding(s)	Irregular shape of the sternum was seen in male and female rats dosed at the top dose, both in groups killed at term or after 2-week withdrawal
Staining	H.E.

Speaker Abstracts

S15: Animal models for Duchenne muscular dystrophy. From pathogenesis to therapy.

Thibaut Larcher
UMR703, INRA – Oniris, Nantes, France

The muscular dystrophies represent a heterogeneous group of muscle degenerative genetic disorders characterized by progressive waste of muscle mass with no curative therapy to date. The most common and the most severe form of the disease is the X-linked Duchenne Muscular Dystrophy (DMD), a recessive disorder affecting approximately 1 of 3,500 to 7,500 newborn live human males¹. The underlying cause is a mutation on the dystrophin gene that results in a complete lack of the protein at the fiber membrane of skeletal and cardiac muscles². Cycles of degeneration and regeneration occur until loss of regenerative capacities of the tissue and consecutive replacement of muscle fibers by fibrosis and adipose cells. Clinical progression of DMD is devastating. From about 4 years, affected boys begin to have trouble. Decreased motility and weakness ensue, confining to wheel chairs before 14 years of age. Severe cardiac abnormalities and respiratory failure lead ultimately to death in their third decade.

It can be assumed that any animal that shows dystrophin deficiency in muscle with a X-linked inheritance, can be a true animal model of DMD if key hallmarks of the human disease are reiterated in a reproducible manner. As dystrophin is very highly conserved through animal evolution, it is not surprising that such genetic and molecular homologues have been identified in several animal species including mouse, dog, cat, fish and invertebrates.

Mdx mouse (X-linked muscular dystrophy) is the most extensively studied model and large parts of our understanding of the molecular mechanisms that underlie the pathogenesis of DMD can be attributed to studies on it. The original mdx mutant contains a premature stop codon in exon 23 responsible for the absence of dystrophin. Some mdx variants like mdx4cv and mdx5cv were additionally produced by mutagenesis, each containing a different mutation leading to a loss of dystrophin protein expression in skeletal muscles³. Histologically, muscles of mdx and variants reproduce primary lesions of myopathy including myofiber necrosis and regeneration. Compared to patients, waves of muscle fiber degeneration are mainly observed at a young age, decreasing in adulthood and levels of regeneration remaining elevated. Consecutively, secondary lesions represented by loss of muscle tissue and fibrosis, is less pronounced than in DMD patients with the exception of the diaphragm muscle⁴. The clinical phenotype is also much more benign than that of boys with DMD: life span is only slightly shorter as compared to wild type controls⁵ and muscle weakness in cage-reared animals is not evident. Muscle pathology in the mdx mouse can be aggravated by forced exercise. Plasma creatine kinase levels are further elevated, forelimb strength is generally reduced and fiber necrosis increased. In this worsen phenotype background, efficacy of an experimental therapy can further be evaluated more accurately⁶.

Emergence of new molecular strategies targeting specific gene mutation stresses the need of “humanized” models to assess efficacy and safety. In this context, exon 52 was disrupted to create a new mouse model with large deletions in the dystrophin gene like those found in two thirds of human patients⁷. Overall, the dystrophic phenotype of these mdx52 mice is similar to age-matched mdx but few data are still available on disease progression. Others described the integration of an intact and functional copy of the entire human dystrophin gene into a mouse chromosome with no phenotypic consequence⁸.

As the attenuated phenotype of dystrophic mice may be explained by over-expression of the dystrophin orthologue, utrophin, or by high muscular regeneration capacities, some teams targeted both dystrophin and utrophin or both dystrophin and telomerase activity in an attempt to aggravate the pathology of the model. These double mutant mice exhibit a markedly reduced life span, severe muscle weakness with joint contractures, and progressive muscle degeneration with interstitial fibrosis. These disease features share many phenotypical hallmarks with DMD but cannot represent a genetic homolog of the human disease⁹.

Numerous dog breeds with dystrophin-deficient muscular dystrophy have been characterized clinically, but few have been studied at the molecular level. Muscular dystrophic Golden Retriever dog (GRMD), the second most extensively studied animal model after mdx, exhibits clinical signs and muscle changes far more closer to its human pathological counterpart. Lack of dystrophin in GRMD dogs is due to a single base change in the 3' consensus splice site of intron 6, leading to skipping of exon 7 and alteration of the reading frame in exon 8, which creates a premature stop. Clinical course of the

Speaker Abstracts

disease may vary considerably between individuals with the same mutation and this variability must be considered when establishing functional endpoints. Some pups survive only for a few days displaying severe selective muscle necrosis (i.e. tongue, masticatory and trunk muscles), many young GRMD dogs die from respiratory failure or complications from digestive troubles (megaesophagus, hiatal hernia) before the age of one year while others are still ambulant for months or even years¹⁰. Modifier genes may play a role in disease pathogenesis and it has now been recognized an ameliorating effects of outbreeding: Labrador retrievers and Beagle dogs with the GRMD mutation survive longer and present a less severe phenotype^{11,12}. Though confounding statistical analysis in preclinical trials, these phenotype variations reproduce what is observed in patients.

From birth, creatine kinase levels are elevated in affected dogs. They develop a progressive muscular weakness and gait abnormalities from the age of 6 to 9 weeks. Typically, they show a more stilted gait, lumbar kyphosis and a plantigrade stance due to hyperextension of the carpal joints and flexion of the tibiotarsal joints. They also develop early pharyngeal and esophageal dysfunctions with excessive drooling and their respiratory capacity is decreased. Most severely affected dogs have difficulties to rise and can walk only a few steps¹⁰. At the light microscopic level, there is an spatial association of elementary lesions that is typical of dystrophin-deficient muscular dystrophy across species. Necrosis of small groups of myofibers that appear swollen and hyaline, fragmented or mineralized, elicits some mononuclear cell infiltration. Clusters of numerous small regenerating fibers are also present. Association of hypertrophic fibers and small regenerative ones is responsible for anisocytosis. The main interstitial modification is extensive endo- and peri-mysial fibrosis, sometimes admixed with some adipose tissue infiltration. Given the overall similarities between affected boys and GRMD dogs and the closeness of their body mass, this model has found favour in recent years, especially in preclinical evaluation of gene and cell therapies for which assessment of tissular distribution of the therapeutic agent and its fate is critical.

The only other natural dystrophin deletion described in a mammalian model occurs in the dystrophic cat. The so-called hypertrophic feline muscular dystrophy (hfmd) model is characterized by extensive muscle hypertrophy. Affected cats eventually die due to compression of the esophagus by the hypertrophied diaphragm or because of impaired water intake caused by glossal hypertrophy¹³. As it lacks the hallmarks of generalized muscle wasting, this model does not mimic DMD as closely as the GRMD dog. Non-mammalian dystrophin-deficient animal models such as zebrafish and the nematode *C. elegans*, can represent an attractive alternative as they can be maintained in large numbers, and are readily genetically manipulable. This has led to their use in both DMD-related gene analysis and high-throughput drug discovery studies¹⁴.

The two most studied models were extensively used to test new therapeutic approaches. Efficacy assessment requires specific tests fitted to the dystrophin-deficient model, *i.e.* its size and its phenotype, and most clinical tests developed for patients cannot be used on animals. We and others have developed various tests to objectively characterize disease progression. Composite scores that take into account numerical values assigned to particular clinical signs or overall disease phenotype, have been described and used in preclinical GRMD trials. Biomarkers of myopathy can also be evaluated in animal models like muscular strength, serum level of muscular intracytoplasmic molecules that leak from the damaged myofiber, tissular imaging and histopathological quantitative assessment of muscular samples. New tools will undoubtedly emerge and will be integrated in a sensitive multiparametric test taking into account interindividual variability.

Some efforts regarding protocol standardization have been made to ensure translation of results obtained on animal models for clinical trials. Several studies have provided largely general proof-of-concept using one of these animal models. Successful treatment may eventually be identified through the use of several of them, differentially suited to addressing specific questions.

1. Emery AE: The muscular dystrophies, *Lancet* 2002, 359:687-695
2. Hoffman EP, Brown RH, Jr., Kunkel LM: Dystrophin: the protein product of the Duchenne muscular dystrophy locus, *Cell* 1987, 51:919-928
3. Chapman VM, Miller DR, Armstrong D, Caskey CT: Recovery of induced mutations for X chromosome-linked muscular dystrophy in mice, *Proc Natl Acad Sci U S A* 1989, 86:1292-1296
4. Connolly AM, Keeling RM, Mehta S, Pestronk A, Sanes JR: Three mouse models of muscular dystrophy: the natural history of strength and fatigue in dystrophin-, dystrophin/utrophin-, and laminin alpha2-deficient mice., *Neuromuscul Disord* 2001, 11:703-712
5. Chamberlain JS, Metzger J, Reyes M, Townsend D, Faulkner JA: Dystrophin-deficient mdx mice display a reduced life span and are susceptible to spontaneous rhabdomyosarcoma, *FASEB J* 2007, 21:2195-2204
6. Willmann R, Possekel S, Dubach-Powell J, Meier T, Ruegg MA: Mammalian animal models for Duchenne muscular dystrophy, *Neuromuscul Disord* 2009, 19:241-249

Speaker Abstracts

7. Araki E, Nakamura K, Nakao K, Kameya S, Kobayashi O, Nonaka I, Kobayashi T, Katsuki M: Targeted disruption of exon 52 in the mouse dystrophin gene induced muscle degeneration similar to that observed in Duchenne muscular dystrophy, *Biochem Biophys Res Commun* 1997, 238:492-497
8. t Hoen PA, de Meijer EJ, Boer JM, Vossen RH, Turk R, Maatman RG, Davies KE, van Ommen GJ, van Deutekom JC, den Dunnen JT: Generation and characterization of transgenic mice with the full-length human DMD gene, *J Biol Chem* 2008, 283:5899-5907
9. Banks GB, Chamberlain JS: The value of mammalian models for duchenne muscular dystrophy in developing therapeutic strategies, *Curr Top Dev Biol* 2008, 84:431-453
10. Kornegay JN, Bogan JR, Bogan DJ, Childers MK, Li J, Nghiem P, Detwiler DA, Larsen CA, Grange RW, Bhavaraju-Sanka RK, Tou S, Keene BP, Howard JF, Wang J, Fan Z, Schatzberg SJ, Styner MA, Flanigan KM, Xiao X, Hoffman EP: Canine models of Duchenne muscular dystrophy and their use in therapeutic strategies, *Mamm Genome* 2012, 23:85-108
11. Miyazato LG, Moraes JR, Beretta DC, Kornegay JN: Muscular dystrophy in dogs: does the crossing of breeds influence disease phenotype?, *Vet Pathol* 2011, 48:655-662
12. Shimatsu Y, Katagiri K, Furuta T, Nakura M, Tanioka Y, Yuasa K, Tomohiro M, Kornegay JN, Nonaka I, Takeda S: Canine X-linked muscular dystrophy in Japan (CXMD)), *Exp Anim* 2003, 52:93-97
13. Gaschen FP, Hoffman EP, Gorospe JR, Uhl EW, Senior DF, Cardinet GH, 3rd, Pearce LK: Dystrophin deficiency causes lethal muscle hypertrophy in cats, *J Neurol Sci* 1992, 110:149-159
14. Collins CA, Morgan JE: Duchenne's muscular dystrophy: animal models used to investigate pathogenesis and develop therapeutic strategies, *Int J Exp Pathol* 2003, 84:165-172

Speaker Abstracts

S16: Stem cell-based therapy for DMD: current clinical experimentation and novel pre-clinical strategies

Francesco Saverio Tedesco^{1,2}, Fabio Ciceri², Yvan Torrente³, Stefano Previtali⁴, Sara Benedetti², Hidetoshi Hoshiya¹, Mattia Gerli², Rossana Tonlorenzi⁴, Mitsuo Oshimura⁵ and Giulio Cossu^{1,2}

¹Department of Cell and Developmental Biology and Centre for Stem Cells and Regenerative Medicine, University College London, UK

²Division of Regenerative Medicine, Stem Cells and Gene Therapy, San Raffaele Hospital, Milan, Italy

³Department of Neurological Science, University of Milan, Policlinico Mangiagalli-Regina Elena, Milan, Italy

⁴Institute of Experimental Neurology, San Raffaele Hospital, Milan, Italy

⁵Institute of Regenerative Medicine and Biofunction, Tottori University, Yonago 683-8503, Japan.

Duchenne muscular dystrophy (DMD) is caused by mutations in the gene encoding the protein dystrophin (1,2). DMD primarily affects skeletal muscles, resulting in progressive paralysis and premature death (3). There is no successful treatment, but new strategies are under investigation (4). Gene and cell therapy of DMD is complex because of the large size of dystrophin gene (2.4Mb) and cDNA (14Kb). In recent years, intra-arterial mesoangioblast (MAB: vessel-associated stem/progenitor cells) transplantation caused amelioration of DMD animal models (4): this was mainly due to their ability to cross the vessel-wall (an advantage over canonical myogenic progenitors such as satellite cells and myoblasts). Cells similar to MABs were isolated from human skeletal muscle and are currently under clinical experimentation for a Phase I/II trial based upon four consecutive intra-arterial infusions of HLA-matched donor-derived MABs (5). While safety is the primary objective of the study, a possible modification in patients' muscle function is also being measured.

Autologous transfer of genetically corrected cells would be desirable, since it would not require immune-suppression. To this end, we reported recently the amelioration of a model of DMD by a novel strategy that combines Human Artificial Chromosome (HAC)-mediated dystrophin gene-replacement with mouse MAB transplantation (5). Indeed, at variance with conventional gene therapy vectors, HACs can carry large genetic regions (e.g. containing an entire locus) and remain episomal (6). We are currently extending this approach to human skeletal muscle derived and pluripotent stem cell derived MABs (7), engineering next generation HACs for future clinical translation of this autologous strategy for DMD.

References

1. E. P. Hoffman et al., *Cell* 51, 919–928 (1987).
2. M. Koenig et al., *Cell* 50, 509–517 (1987).
3. A. E. Emery, *Lancet* 359, 687–695 (2002).
4. F. S. Tedesco et al., *J. Clin. Invest.* 120, 11–19 (2010).
5. F. S. Tedesco et al., *Science Transl. Med.* 3, 96ra78 (2011).
6. Kazuki, M. Oshimura, *Mol. Ther.* 10.1038/mt.2011.136 (2011).
7. F. S. Tedesco et al., *Science Transl Med* 4, 140ra89 (2012).

Research funding

EC, ERC, MRC, Telethon, Duchenne Parent Project and Italian Ministries of Research and Health.

Speaker Abstracts

S17: Pathology endpoints in routine repeated dose toxicology studies: A review of global regulations

Ken Schafer

Vet Path Services, Inc., Mason, Ohio, USA

The reason for the selection of the various pathology parameters for a routine, repeat dose toxicity study is not always apparent to the casual observer, even those who are pathologists. Many of the parameters evaluated are specifically addressed in various regulatory guidances. Others are recommended by white papers from toxicologic pathology societies. Some parameters are evaluated with a scientific basis, while others have the appearance of filling a need for “box checking”. This presentation focuses on the parameters often evaluated in routine toxicology studies in the pathology arena, including clinical pathology, organs and tissues evaluated grossly at necropsy, organ weights, and histopathology. The regulatory guidances, primarily of pharmaceutical regulatory bodies, that specifically address the areas of pathology will be discussed. These include those from North America, Europe, and Japan, as well as ICH guidances. In addition, recommended practices from the societies of toxicologic pathology will also be covered where appropriate.

Speaker Abstracts

S18: Pathology Peer Review in preclinical safety studies – international perspectives, recommendations, regulatory issues

Erio Barale-Thomas
Janssen R&D, Beerse, Belgium

A brief chronicle of pathology peer review

This part will present how the concept and practices of PR evolved since FDA implemented GLP, based on a full bibliographic listing which is mostly summarized in the papers below. This will allow to understand some of the current controversies with the regulatory authorities.

Morton D, et al., Recommendations for Peer Review. *Toxicol Pathol* 2010, 38:1118-27

McKay JS, et al. Commentary on the Process of Peer Review and Pathology Data Locking *Toxicol Pathol* 2010, 38: 508 510
JSTP. Panel Discussion: Regulatory perspective for pathology data. *J Toxicol Pathol* 2009, 22:209 27

Crissman JW, et al. Best Practices Guideline: Toxicologic Histopathology. *Toxicol Pathol*, 2004, 32:126-131

Summary of the STP paper “Recommendations for Pathology Peer Review”

This concise summary of the recent paper by Morton will present the “state-of-the-art” peer review as recommended by the North-American STP and endorsed by other STPs. Specific topics will be discussed by other presenters (see the program).

Controversial topics:

Several controversial topics will be examined, among others:

- The study protocol / amendment and the details of the peer review process
- The type of studies to peer review
- The target organs in a tox study
- The proliferative and neoplastic changes in carcinogenicity studies
- The extent of the PR documentation
- The timing of the signature as a proof of traceability, personal responsibility and interaction without undue influence
- The interaction between the industry and the CROs, particularly with the 2 previous points
- The start of locking and audit-trailing the pathology data vis-a-vis the draft pathology report and the PR
- PR using digital images
- The PR-related material to retain and to archive with study file / raw data

Initiatives of the regulatory agencies

The recent FDA statements during CROs audits will be examined in view of the adaptations of the process that they suggest. The progress of the OECD working group on pathology peer review towards the issuance of an advisory document will be also presented.

Speaker Abstracts

S19: Peer Review in Toxicologic Pathology – current situation in Japan

Takanori Harada, Nishikawa A, Oishi Y, Suzuki M, Teranishi M, Yoshida M, Yoshizawa K, and Mitsumori K
Peer Review Ad hoc Working Group, The Japanese Society of Toxicologic Pathology

It is generally accepted that pathology peer review (PPR) is an important procedure to verify and improve the accuracy and quality of histopathology data generated by the study pathologist in toxicological studies of xenobiotics including pharmaceuticals and agrochemicals with experimental animals.

However, the timing of PPR relative to data locking and the definition of raw data differ among countries. In the United States and Europe, PPR is usually conducted before pathology data are fixed or locked. In Japan, internal PPR within the testing facility is conducted before data locking, but sponsor PPR is mostly performed after pathology findings are fixed, which is in line with the guidance described in the GLP Guidebook 2006 that is recommended by the Japanese representative regulatory agency, Pharmaceuticals and Medical Devices Agency (PMDA). The purpose of the guidance by PMDA appears to ensure transparency of PPR process and to minimize the influence of sponsor power on the original data by the study pathologist. In order to clarify differences in definition of pathology raw data and peer review process among countries, a Panel Discussion on Regulatory Perspective for Pathology Data was held during the 25th Annual Meeting of the Japanese Society of Toxicologic Pathology (JSTP) at Hamamatsu in 2009. The panelists who participated in the discussion represented multiple societies of toxicologic pathology including the JSTP, Society of Toxicologic Pathology (STP), and European Society of Toxicologic Pathology (ESTP) together with the Japanese Society of Quality Assurance (JSQA). It was also cooperated by the International Federation of Societies of Toxicologic Pathology (IFSTP) and its Regulatory Interaction Committee (RIC) for assistance in pursuing a globally acceptable approach to peer review. At that meeting, it was revealed that USA and European regulatory agencies do not request that data be locked before peer review or an audit trail of changes in the pathology report be produced, which is different from that in Japan. Since international harmonization of PPR practices is very important, the JSTP and other relevant parties including JSQA had a face to face meeting with PMDA in 2010 to discuss the differences between Japan and other countries and also the content of a draft OECD guidance on pathology peer review. At the meeting, the JSTP recommended to PMDA that PPR prior to data locking would be more suitable to improve the quality and reliability of pathology data to be submitted to regulatory agencies. The JSTP position is consistent with that of STP which is shown in “Recommendations for Pathology Peer Review” by the STP Peer Review Working Group published in *Toxicologic Pathology* in 2010. PMDA also gathered information on PPR in other countries as much as possible. Taken together, PMDA showed new draft viewpoints on PPR at the 3rd Global Quality Assurance Conference which was held at Kyoto in 2011. The PMDA’s draft viewpoints are summarized as follows:

- PPR is not mandatory to nonclinical studies of pharmaceutical products, but if PPR is carried out, then it is subject to GLP inspection.
- At the moment, PMDA considers that pathology raw data is the report or data signed and dated by the study pathologist.
- PPR before or after data locking would be acceptable either way, but PPR by pathologists outside from sponsors or academia may be required to ensure the transparency of review process and to be described in the protocol if the conduct is scheduled in advance.
- In addition, the name of the pathology peer reviewer and reviewed organs with disagreement should be described in the final report.
- The report or data generated by the peer reviewer should be archived together with the other study documents.

The JSTP basically agree with the new draft viewpoints of PMDA, although we need further discussions in details before it is finalized.

Speaker Abstracts

S20: Pathology Working Groups (PWGs): definition, contexts of application in toxicity and carcinogenicity studies, and examples

Jerry F. Hardisty

Experimental Pathology Laboratories, Inc., Research Triangle Park, NC, USA

The Pathology Working Group (PWG) is a specialized type of review. Unlike the routine peer reviews, PWGs are convened to answer specific questions regarding study results. They may be convened by study sponsors, consortiums or government agencies. PWGs are generally conducted after a study (or studies) have been finalized, and thus require full documentation.

The panel for a PWG is composed of a group of expert pathologists who are assembled to discuss a specific question regarding study results. Since the purpose of the PWG is to provide an independent unbiased opinion, members of the PWG may come from academia, government or industry. Panel members are selected based on their experience in toxicologic pathology, as well as their expertise in the particular area being discussed. Both veterinary and medical pathologists with appropriate expertise may serve as members of a PWG.

Pathology Working Groups may be convened to answer any number of specific questions. Some possible situations where a PWG might be useful include:

- Studies with final reports
- Pivotal studies with controversial end points
- Address questions that are of concern by regulatory agencies
- Comparison of results of multiple studies that may have been conducted and evaluated by different laboratories and/or pathologists

Although PWGs are most often convened to discuss tumor endpoints (diagnostic criteria for different tumor types and the impact of this on relationship to treatment), they can also be convened to discuss the incidence and severity of non-neoplastic findings. PWGs for non-neoplastic findings are often initiated when studies have been evaluated at several different institutions, and there is a lack of clarity regarding diagnostic criteria or consistency in severity grading.

Speaker Abstracts

S21: Interactions between Sponsors and CRO pathologists: What can be improved?

Kathleen Funk and Jeff Engelhardt
Experimental Pathology Laboratories, Inc., Sterling, VA USA

The pathology report is often the pivotal evaluation used by sponsors and health authorities to make safety decisions regarding a test article. The quality of the pathology report depends on the data generated in the study and the perspective provided by the pathologist evaluating and interpreting those data. Achieving appropriate identification and perspective on potential test article-related lesions requires openness in communication between a sponsor and the CRO pathologist. Without knowledge of the pharmacologic action, class of chemical or biological agent, or what has been observed in previous toxicity or PKDM studies places the CRO study pathologist at a disadvantage to fully evaluate the study at hand. Without this knowledge, the pathologist may misinterpret subtle histological alterations or introduce new terminology for test article-related lesions. Neither occurrence assists a sponsor or health authority to make informed decisions regarding safety. The pathology report should be sufficiently descriptive so the test article-related alterations are clearly defined and the rationale for what alterations are not due to the test article is fully presented. Interpretive comments on potential pathogenesis of alterations should be supported by the study data and not be speculative in nature. Similarly, adversity should be determined solely from the study data for collective findings in an organ or tissue and not for individual findings. Review of the draft pathology report by the sponsor should focus on the scientific content and not on the writing style. Collated comments on the report should be sent to the study pathologist in a timely manner. It is also important that blinded histopathology evaluation and pathology peer review is incorporated appropriately and that a contingency plan to evaluate intermediate dose groups be developed. In the end, a pathology report following these suggestions will be of the best quality for review by a sponsor or health authority assessor to make judgment on the safety of the test article.

Speaker Abstracts

S22: Industry-CROs Pathology interactions – a sponsor's perspective

Armando R. Irizarry Rovira

Eli Lilly and Company, Indianapolis, Indiana, USA

The pharmaceutical industry is dynamic and continuously changes the process by which it achieves the ultimate goal of delivering life-saving or life-improving medicines to patients. Accurate nonclinical data demonstrating safety are a cornerstone of this process. In recent years there has been a larger shift by pharmaceutical companies to outsource preclinical toxicology studies to Contract Research Organizations (CROs). Although these outsourced studies are not conducted at the pharmaceutical Sponsor's facilities, the Sponsor has an inherent stake and responsibility in ensuring that nonclinical toxicology data is accurate and of high quality. A key component of nonclinical toxicology studies is the generation and interpretation of pathology data. There is a genuine interest on the part of both the CROs and Sponsors of these studies in ensuring that the pathology data is accurate and that the reports are of high quality. CROs and sponsors must collaborate to balance each other's needs and at the same time generate high quality data. The success of this collaborative relationship will depend on thorough communication at all stages of a toxicology study, including the pathology peer review process. This presentation will provide a Sponsor's point of view on practices which facilitate the creation of pathology reports of high quality. The discussion will be limited to topics that impact the pathology contributor report of toxicology studies covered by Good Laboratory Practices (GLP).

References

1. Irizarry Rovira A, Foley GL, Clemo FA. (2011). Sponsor-CRO Practices That Facilitate the Creation of a High-Quality Pathology Report: A Pharmaceutical Sponsor's Perspective. *Toxicologic Pathology*, 39:1013-1016.
2. Gosselin, S.J., Palate, B., Parker, G.A., Engelhardt, J.A., Hardisty, J.F., McDorman, K.S., Tellier, P.A., and Silverman, L.R. (2011). Industry – contract research organization pathology interactions: a perspective of CROs in producing the best quality pathology report. *Toxicol Pathol* 39:422-428.
3. Kopplin JR, and Richter WR. (2007). Selection and utilization of CROs for safety assessment. In *Preclinical Development Handbook: Toxicology* (S.C. Gad ed.). pp. 979-991. John Wiley and Sons, Inc., Hoboken, NJ, USA.

INHAND Poster

INHAND Poster 1: INHAND Update (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice)

Keenan, C, Baker, J, Bradley, A, Kaufmann, W, Nolte, T, Rittinghausen, S, Kellner, R, Harada, T, Herbert, R, Tanaka, T, Goodman, D, Mann, P, Vahle, J, Mahler, B

Harmonization of nomenclature and diagnostic criteria in toxicologic pathology, especially for rats and mice, has been a goal of pathologists working in the profession for many years. In the latter part of the twentieth century, several initiatives were undertaken by the STP in the United States and by the RITA data base group (Registry of Industrial Toxicology Animal-data) in Europe. Their efforts resulted in a number of internationally recognized publications: SSNDC: Guides for Toxicologic Pathology and the WHO/IARC International Classification of Rodent Tumors. Beginning in 2005, the STP and European Society of Toxicologic Pathology (ESTP), in conjunction with RITA, developed a collaborative process to review, update, and harmonize existing nomenclature documents and databases. In 2006, the British Society of Toxicologic Pathology (BSTP) and the Japanese Society of Toxicologic Pathology (JSTP) joined the initiative, so that the project has become truly global. The result of these discussions was the INHAND Proposal (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice). A Global Editorial Steering Committee (GESC) oversees the activities of the project. The GESC is composed of toxicologic pathologists from all of the participating societies. In addition there are several technical consultants for web and print support. The Organ System Working Groups (OWG) are the core of the project. Each Group is responsible for producing the non-neoplastic nomenclature for their particular organ. In addition, they review the proliferative lesions already posted on goRENI and discuss them with the RITA groups so that the final nomenclature, both proliferative and non-proliferative represent the consensus of the international collaborators. The Chair of each OWG is appointed by the GESC. Members of the group come from each of the participating Societies and may be nominated by the Chair or by the individual STPs. There are currently active OWGs for all organ systems. The objectives are to produce publications for each organ system that provide a standardized nomenclature and differential diagnosis for classifying microscopic lesions observed in laboratory rats and mice in toxicity and carcinogenicity studies.

INHAND Poster

INHAND Poster 2: Proposed Bone Nomenclature

Healy L, High W, Vahle J, Rinke M, Ernst H, Boyce R, Schelling S, Long P, Boyle M, Bolon B, Fossey S, Bendele A, Jolette J, Roth D, and Leininger J.¹

¹INHAND Skeletal Nomenclature Committee

The INHAND Project (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) is a joint initiative of the Societies of Toxicologic Pathology from Europe (ESTP), Great Britain (BSTP), Japan (JSTP) and North America (STP) to develop an internationally-accepted nomenclature for non-proliferative and proliferative lesions in rodents.

The purpose of our work is to provide an update on the status of the standardized nomenclature for classifying lesions observed in bone of rodents. This poster will focus on bone nomenclature; joint and teeth are covered separately.

Proposed Nomenclature: Non-proliferative Lesions

- Fibro-Osseous Lesion (FOL) - Mice only -
- Fibrous Osteodystrophy/Renal Osteodystrophy
- Increased Bone Mass, Trabeculae and/or Cortex, Focal or Diffuse (Preferred over Hyperostosis) Hyperosteoidosis
- Increased Osteoclasts
- Atrophy (Preferred over Osteopenia/Osteoporosis)
- Bone Cyst
- Necrosis
- Fracture/Callus
- Physeal Hypertrophy/Dysplasia

Proposed Nomenclature: Proliferative Lesions

- Osteoblast Hyperplasia
- Osteoma
- Osteoblastoma
- Osteofibroma
- Osteosarcoma
- Fibrosarcoma
- Chondroma
- Chondrosarcoma
- Chordoma, Benign
- Chordoma, Malignant

INHAND Poster

INHAND Poster 3: Proposed Joint and Tooth Nomenclature

Long P, Ernst H, Schelling S, Healy L, High W, Vahle J, Rinke M, Boyce R, Boyle M, Bolon B, Fossey S, Bendele A, Jolette J, Roth D, and Leininger J.¹

¹INHAND Skeletal Nomenclature Committee

The INHAND Project (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) is a joint initiative of the Societies of Toxicologic Pathology from Europe (ESTP), Great Britain (BSTP), Japan (JSTP) and North America (STP) to develop an internationally-accepted nomenclature for non-proliferative and proliferative lesions in rodents.

The purpose of our work is to provide an update on the current status of the standardized nomenclature for classifying lesions observed in joint and teeth of rodents. This poster will focus on joint and teeth nomenclature, bone is covered separately.

Joint: Proposed Nomenclature - Non-proliferative Lesions

- Osteophyte (Chondrophyte)
- Inflammation
- Chondromucinous Degeneration.
Degenerative Joint Disease

Joint: Proposed Nomenclature - Proliferative Lesions

- Synovial Cell Hyperplasia
- Synovial Sarcoma

Tooth: Proposed Nomenclature - Non-proliferative Lesions

- Degeneration, Ameloblasts
- Degeneration, Odontoblasts
- Necrosis
- Dentin Niches
- Dentin, Decreased
- Dentin Matrix Alteration
- Dysplasia
- Fracture
- Resorption
- Ankylosis
- Denticles
- Pulp Stone
- Cyst
- Thrombus

Tooth: Proposed Nomenclature - Proliferative Lesions

- Odontoma, Complex
- Odontoma, Compound
- Odontoma, Ameloblastic
Ameloblastoma
- Fibro-Odontoma, Ameloblastic (Mouse Only)
- Fibroma, Odontogenic
- Fibroma, Cementifying/Ossifying
- Tumor, Odontogenic, Benign
- Tumor, Odontogenic, Malignant

Poster Abstracts

P01: Safety of Oncolytic Measles-Virus-SuperCD in Transgenic Mice and Rhesus Macaque

Iris Völker¹, Patricia Bach¹, Cheick Coulibaly², Roland Plesker², Tobias Abel¹, Michael Mühlebach³, Ulrich Lauer⁴, Christian Buchholz¹

¹Molecular Biotechnology and Gene Therapy

²Animal Facilities

³Oncolytic viruses and Vaccines, Paul-Ehrlich-Institut, Germany

⁴Department of Gastroenterology and Hepatology, Medical University Hospital, Tübingen, Germany

Background

Liver cancer is a common cancer type, but conventional treatment options are often unsatisfactory. Now, viruses which destroy cancer cells represent a new class of therapeutic agents. Oncolytic measles viruses (MV) already entered clinical phase. They can be armed, targeted or shielded e.g. by transgenes. MV-SuperCD is a live attenuated vaccine strain, encoding the suicide gene SuperCD, which is a combination of the yeast cytosine deaminase and the uracil phosphoribosyltransferase genes. It converts the prodrug 5'fluorocytosine into the chemotherapeutic compound 5'fluorouracil. This is supposed to enhance tumor cell killing. However, the safety profile of MV-SuperCD when injected intrahepatically (ih) has to be assessed in two animal models.

Methods

Biodistribution, shedding and toxicology studies of MV-SuperCD were performed in MV-susceptible IFNAR^{-/-}-CD46^{Ge} mice. A single low dose of MV-SuperCD was injected ih after laparotomy or at high dose into the peritoneal cavity (ip), followed by prodrug administrations two days later. Blood samples and necropsies were analyzed on four time points up to day 93. Toxicology studies in rhesus macaques followed for ih application.

Results

Clinically, the treatment was well tolerated. Quantitative real time PCR demonstrated presence of MV-N gene in various organs that declined later. No infectious virus was shed as confirmed by titration on Vero cells. When a control GFP-encoding MV was injected ip, GFP-expression was detected in many organs by histology. Stable anti-MV-N antibodies were induced. Blood parameters including liver enzymes revealed no deviations. Histological analyses of organs were evaluated for adverse effects. Finally, hepatocytes were cultivated from control animals, infected with MV-SuperCD and stained by immunofluorescence.

Conclusions

The tested dose was 420-fold above a potential clinical dose demonstrating a high level of safety for MV-SuperCD. Taken together, these data indicate that MV-SuperCD is safe upon single ih injection for treatment of liver cancer.

Poster Abstracts

Po2: When a biologics drug targets a viral disease: From TCR validation to off-target staining

T. Flandre, V Thoree, S Jones, A Hey

PCS Project Pathology and BxSD - Novartis Institutes for Biomedical Research, Basel, Switzerland

A Tissue Cross-Reactivity (TCR) study was developed and run to characterize, using immunohistochemical (IHC) techniques, the potential cross-reactivity of a monoclonal antibody (ABC123) intended to treat disease caused by a virus from the Herpesviridae family (HVF).

Method development was done on preparations of HVF infected human cells as positive control material and non-infected wild type cells as negative control. To validate the method, an isotype control and ABC123 were tested at different concentrations with different IHC protocol on the negative and positive material.

During method development, infected cells were showing positive staining with ABC123 but also with isotype. Virus from Herpesviridae family are producing a viral glycoprotein FcR like (Lubinski et al. 1998) that binds the Fc part of any IgG. To prove specificity of ABC123, Fab fragments from isotype and ABC123 were produced and applied on infected cells. Positive staining was still present with the Fab portion of ABC123 whilst the Fab portion of the isotype was negative as expected.

The final step was to validate the method on the few tissues known to be negative or positive for HVF. A specific positive staining was observed in scattered cells from positive HVF tissues. This staining was confirmed by in situ hybridization (ISH) using a commercial probe detecting the presence of HVF DNA/RNA in tissues. This ISH method was developed to assist in the interpretation of any on- or off-target binding observed with ABC123 since the seropositivity status of human tissues used in this study was generally unknown.

During the TCR, scattered positively stained cells were observed in different organs of some donors with ABC123. The observed staining pattern in these tissues was consistent with the expected profile of HVF infection in human cells; this was further confirmed by the presence of HVF DNA/RNA using ISH techniques. In the meantime diffuse staining was observed in keratinized cells of the stratum spinosum/granulosum/corneum in the skin and of the Hassall's corpuscles in the thymus of all donors.

When this type of unexpected staining is observed, three questions are raised. Is it background staining? Is it on-target (massive infection by HVF)? Is it off-target binding? No staining or background staining was observed with isotype or a second antibody DEF123 directed against a different epitope. This diffuse staining was only observed with ABC123 and ISH was negative. By the way, this staining was considered to be off-target with ABC123 binding to a protein present in/on human keratinocyte like cells. Absence of staining in the skin and thymus with DEF123 was confirming the off-target binding of ABC123 in these tissues.

Further investigation showed that blasted, epitope of ABC123 presents less than 60% homology with human proteins. Current investigations of the protein binding ABC123 have fished a ~50 kDa protein which will need further identification and colocalization.

In conclusion, this study has shown the relevance and value of completing TCR studies for biologics products when it is technically feasible. This means the most appropriate positive control might be characterised according to the target (infected cells) to allow a robust method development with low signal:noise ratio. Nevertheless, new techniques need to be further developed and improved to assist confirmation of the staining as cytoplasmic and/or membranous, and might help in risk assessment of the relevance of off-target binding to a cytoplasmic protein.

Lubinski J, Nagashunmugam T, Friedman HM (1998) Viral interference with antibody and complement. *Semin.Cell Dev.Biol.*; 9 (3):329-337

Poster Abstracts

P03: Evaluation of an antibody drug conjugate (ADC) biodistribution in a selection of mouse tissues using an immunohistochemistry method

*Olivier Dorchies, Valérie Sallez, Xavier Palazzi, Marie-Laure Ozoux, Gérard Sanderink
Sanofi recherche & développement, Vitry-sur-Seine, France*

The objective of this immunohistochemistry-based biodistribution study was to provide information about the cellular localization of an antibody-drug conjugate (ADC) within the eye (an identified target organ) in comparison with other tissues. This antibody-drug conjugate consisted of an antibody (human IgG₁ raised against a tumoral antigenic target) linked to a payload drug C (cytotoxic in the present case). ADCs are a new type of targeted therapy used for example in oncology.

For this purpose, CD1 mice (9 to 10 weeks of age at dosing initiation) received control vehicle or ADC in vehicle through a single intravenous administration (30-minute infusion). Mice were divided into one control group and 4 groups of 5 mice/sex/group treated at the same dose level of ADC. Each group was necropsied at a specific time point: on Day 2 for the control group and on Days 2, 4, 8 and 15 for the ADC-treated groups. The following organs were sampled for evaluation of the biodistribution of ADC by immunohistochemistry: eye and Harderian gland, liver, lung and mesenteric lymph node. At necropsy, tissue samples were collected, snap frozen and stored at -80°C. Cryosections were prepared on a freezing microtome (6 µm sections) and mounted on glass slides.

Both conjugated and free drug C (the cytotoxic and antibody may be cleaved *in vivo*) were detected using a biotinylated anti-C antibody, and the human IgG₁ backbone of the ADC (coupled to C and free antibody) was detected using a biotinylated anti-human IgG₁ antibody. The ADC and its isotype were also used on control animal tissues in order to assess the baseline expression of the target in mouse.

ADC biodistribution as assessed by immunohistochemistry demonstrated a positive staining in all tissues examined (eye, Harderian gland, liver, lung and mesenteric lymph node) in ADC-treated groups. The staining intensity for C and human IgG₁ observed in the eyes was overall similar to the one recorded for other tissues, and was present in different ocular structures (cornea, choroid, ciliary body [stroma], retina and sclera). For all tissues examined, the maximum staining was generally observed on Days 2 and/or 4 and then slightly decreased with a different kinetic between C and human IgG₁ specific staining. Indeed, on Day 15, C specific staining was absent or minimal whereas human IgG₁ specific staining was still minimal to moderate for most tissues examined, suggesting the persistence of the IgG₁ backbone in the tissues and the loss of the cytotoxic moiety.

Poster Abstracts

Po4: Quinolone-induced cartilage changes - an old story in young animals?

Christine Ruehl-Fehlert¹, Wolfgang Drommer², and Martin Rosenbruch¹

¹Bayer Pharma AG, Wuppertal, Germany

²School of Veterinary Medicine Hannover, Hannover, Germany

Quinolone-induced primary degenerative cartilage lesions of juvenile animals are known as a classical example of juvenile toxicity for more than 30 years. They are regarded as serious effects since their persistence in the cartilage can cause permanent joint damage. Interest in these changes is still vivid since quinolones have been approved for pediatric treatment of certain infectious diseases. Besides children, other groups at higher risk as for example athletes may be identified in future. Still, the mechanism has not been fully elucidated. Proposed pathomechanisms do not foreclose each other but seem to refer to different aspects and events in pathogenesis. Special techniques such as gene expression or electron microscopy for endpoints such as oxidative stress or integrity of the extracellular matrix are useful to compare different compounds and species sensitivity and to obtain additional information on human risk.

Since the findings are highly specific for quinolone toxicity and have been reproduced only by severe magnesium depletion, disruption of magnesium homeostasis seems to be pivotal for the development of lesions.

Histopathology as the classical method of investigation is still valuable since in gene expression studies by Goto et al. (2008), certain profiles coincided with GAG-depletion in the SOFG-stain and changes in ISH-investigations in histological slides. Furthermore, electron microscopy is a valuable tool for the investigation of slight changes which cannot be detected in paraffin sections.

Quinolone arthrotoxicity is generally not species-specific although susceptibility differs among the species with dogs being highly sensitive and humans showing very low sensitivity. Detailed investigation of individual compounds is thus relevant for human risk assessment.

Goto K, Yabe K, Suzuki T et al. (2008) *Toxicology* 249 (2-3): 204 – 213.

Poster Abstracts

P05: A proposal for alternative trimming and inclusion procedure of the knee joint of rodents in toxicity studies

Michele Ardizzone¹, Wolfgang Kaufmann¹, Stephanie Steidler¹, Cristiano Marozin¹, and Alison Bendele²

¹RBM S.p.A - Merck Serono, Colliere Giasosa (TO), Italy

²Bolder BioPATH, USA

Histological examination of rat knee joints in non-clinical toxicity studies is routinely done on parasagittal plane sections. At our laboratories the standard protocol was changed in favour of coronal plane section which gives a better overview of all the anatomical structures of the knee joint. In fact collateral ligaments, capsule (fibrous external layer and synovial membrane), fat pad, menisci (lateral and medial), articular cavity, cruciate ligaments, articular cartilage, tidemark, calcified cartilage, subchondral bone, epiphyseal growth plate, and also bone marrow are well visible and evaluable in a single slide section. The alternative trimming procedure for the coronal plane section is shown and documented with photographs of the relevant phases. The authors believe that this technique offers a number of advantages, mainly including:

- 1) concurrent visualization of the overall knee joint: medial-central-lateral compartments of the joint, medial and lateral menisci, periarticular adnexa (synovia, capsule, collateral ligaments), marginal transitional zones (site of osteophyte formation), cruciate ligaments and their insertions;
- 2) reduced plan section variability among slides;
- 3) evaluation and comparison of the articular cartilage surfaces covered by menisci versus uncovered, with entire visualization of all cartilage layers, from the surface down to the subchondral bone. The coronal plane section method thus allows both medial and lateral compartments of the joint to be shown resulting in improved slides and better characterization of any spontaneous/induced changes in cartilage or subchondral bone.

Poster Abstracts

Po6: Collagen induced arthritis in the common marmosets: A new non-human primate model for chronic arthritis

*Michel P.M Vierboom, Elia Breedveld, Ivanela Kondova, Bert 't Hart
Biomedical Primate Research Centre, Rijswijk, The Netherlands*

The broad immunological gap between inbred specific pathogen free (SPF)-raised rodent strains and the diverse rheumatoid arthritis (RA) patients population limits the predictive value of the current disease models for clinical success of new therapies in particular for those using highly specific biologicals. The close immunological and physiological proximity of non-human primates to patients may bridge the evolutionary gap between rodent models and human and help reduce the problem that many ($\pm 60\%$) new therapies fail in clinical trials.

The established protocol of collagen-induced arthritis (CIA) explored in rodents was used to develop an arthritis model in the New World primate common marmoset (*Callithrix jacchus*). Parameters previously developed in the rhesus monkey model of CIA were used to evaluate the disease course in common marmosets. Overt CIA was observed in all 16 animals. 7 of 16 animals developed predominantly acute CIA, while the rest responded more chronically to induction. Both cellular and humoral responses against Collagen type II were present. Histopathological examination of affected joints detected mild to moderate synovial hyperplasia, forming in some cases pannus tissue overgrowing and eroding the cartilage surface and degrading the subchondral bone. These changes were accompanied by changes in the extra-articular tissues like: inflammation of the subcutis and periosteum, development of perivascularitis, presence of reactive blood vessels and angiogenesis.

The similar disease course observed in several chimeric marmoset twins suggests genetic involvement in the heterogenetic presentation of the disease. This model could be extremely useful in preclinical testing of new human specific therapeutics better predictive for safety and efficacy.

Poster Abstracts

P07: Spontaneous metastatic osteosarcoma in control Sprague-Dawley rats from a 2-year carcinogenicity study – three cases

Francesco Marchesi, Elena Riccardi, and Anna Maria Giusti
Accelera S.r.l., Nerviano (MI), Italy

Reports on the incidence of bone tumors in laboratory rat strains are relatively sparse in the literature. A few comprehensive reviews and classification schemes for bone proliferative lesions have been published. The spectrum of bone neoplasms encompasses benign and malignant tumors, and a number of short reports exist describing cases of osteosarcomas occurring at extra-skeletal sites. Proliferative bone lesions, including osteosarcomas, have been described F344 rats used in chronic toxicity and carcinogenicity studies supporting the nonclinical development program for recombinant human Parathyroid Hormone, Teriparatide [rhPTH (1-34)].

The spontaneous occurrence of malignant bone tumors was evaluated with a retrospective survey on histopathology data of animals from the control groups in a 2-year carcinogenicity study conducted at our facilities.

The results of the survey showed an overall low incidence of malignant bone tumors (osteosarcomas) in Sprague-Dawley rats from the vehicle control groups. Osteosarcoma was diagnosed in 3 out of 50 males, whereas no cases were observed in control females. In one male at final sacrifice (case #1) the osteosarcoma appeared to arise primarily at the level of the rib cage with metastatic deposits noted in the diaphragm, heart, lung and mediastinum. The remaining two cases of osteosarcoma were diagnosed as fatal and identified as contributory causes of death in decedent animals on Days 650 (case #2) and 555 (case #3). In both cases the osteosarcomas showed massive metastatic dissemination to the lung. Notably, in cases #1 and #3 pulmonary metastases appeared more differentiated than the primary tumor, showing the presence of large amounts of variably mineralized osteoid and mature bone tissue in comparison with the histological appearance of the primary neoplasms. In case #3 prominent metastatic involvement of the heart was also observed, with large metastatic deposits affecting the endocardium of the right ventricle, the right atrio-ventricular valve leaflets, and protruding into the ventricular lumen.

Pulmonary metastatic dissemination was a common feature of all three cases of osteosarcoma observed. In particular, in the two animals dying preterm (cases #2 and #3), massive metastatic involvement of the lung (in both cases) and/or of the heart (case #3) likely represented key factors contributing to the lethality of the tumor.

Poster Abstracts

Po8: Background pathology of cynomolgus monkeys (*Macaca fascicularis*) from different origin (Mauritius vs. China/Vietnam)

J.Kaspareit¹, S.Friderichs-Gromoll¹, M.Bleyer², G.Habermann¹, and E. Buse¹.

¹Covance Laboratories, Münster, Germany

²German Primate Center, Göttingen, Germany.

Cynomolgus monkeys (*Macaca fascicularis*) are widely used in toxicological studies required by regulatory authorities for the marketing of new drugs. However, the interpretation of these studies requires an exact knowledge of the background pathology of this species to avoid misinterpretation of lesions seen in test-item treated groups and to be able to differentiate them from naturally occurring pathological changes. Only few studies help the toxicological pathologist up to now to do this job. However, these studies describe lesions in immature animals, wild-caught animals of unknown age or cover only a part of the organ spectrum that is examined in toxicological studies. Furthermore studies comparing background pathology of cynomolgus monkeys of different origin are completely lacking or based on a rather low number of animals.

For this study slides from 320 cynomolgus monkeys (80/sex/origin) were reviewed. Animals were from control groups of 41 toxicological studies (22 with animals of mauritian origin, 19 with animals of chinese/vietnamese origin) conducted between 2003-2011 at our institution. A complete necropsy was performed on all animals after euthanasia with sodium pentobarbitone and exsanguination. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at a nominal thickness of 5µ and stained with hematoxylin and eosin. Mean age and weight and the incidences of common lesions grouped by organ system are presented. Only lesions that occurred in the study more than one time are included in the tables.

It was the objective of the present study to report the background pathology of the primate species (*Macaca fascicularis*) that is most frequently used today in toxicological studies. Furthermore we have concentrated specifically on differences in pathology of primates from the two major sites of origin the great majority of laboratory cynomolgus monkeys are purchased from. Only few pathological lesions were found to have striking differences between both sites of origin. The great majority of changes showed about equal frequency or the number of lesions or was too low for comparison. To our knowledge this is the first study comparing background pathology of a large number of cynomolgus monkeys from the two major sites of breeding. Furthermore, data of mature primates are reported for the first time.

Poster Abstracts

P09: Workflow for establishing historical control data in the RITA database

Rupert Kellner and Susanne Rittinghausen

Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany

The interpretation of results from long-term carcinogenicity bioassays in rodents is facilitated by reliable historical control data. These data need to fulfill a number of criteria including consistency and validity. Therefore, the main effort in maintaining and expanding the historical control database RITA ('Registry of Industrial Toxicology Animal-data') is devoted to meeting these requirements. The RITA database was established in 1988 and is a concerted project of pharmaceutical and chemical companies from Europe and North America. Proliferative lesions found in control groups of carcinogenicity studies in rats and mice are stored on an animal-by-animal basis together with a multitude of background data.

From the beginning, a basic requirement was the definition of standards, e.g. for the trimming guidelines, applied nomenclature, diagnostic criteria, and the data entry process. Diagnostic terms used in the original study report thus need to be translated into the terms used by RITA. Data acquisition has evolved over time and is now performed through encrypted connections on a secure server. Data validation procedures include a peer review of all proliferative lesions by a Fraunhofer pathologist and discussion of selected findings by a panel of experienced pathologists from the member companies. The adoption of INHAND nomenclature poses new challenges to the whole procedure as all steps need to be concordant with this modified terminology.

Poster Abstracts

P10: Digital Image Microscopy Review of Chronic Study PWGs by Pathologists Naïve to the Original Glass Slide Review

Rodney A. Miller¹, Gabrielle A. Willson¹, E. Terence Adams¹, Jerry F. Hardisty¹, Greg R. Olson³, William M. Witt², and David E. Malarkey²

¹Experimental Pathology Laboratories, Inc., RTP, NC

²National Institute of Environmental Health Sciences, RTP, NC

³Toxicologic Pathology Associates, Jefferson, AR, USA

This poster describes one of several experiences using digital image microscopy where a large group of pathologists reviewed digitally scanned slides and the results of their reviews were compared to the original glass slide review. These pathologists had neither prior knowledge of individual diagnoses nor had participated in the glass slide PWG. This review was an exact duplication of glass slide PWGs. Eighty-six percent (86%) of all rat digital consensus diagnoses were in agreement with the glass slide consensus diagnoses. Eighty-four percent (84%) of all mouse digital consensus diagnoses were in agreement with the glass slide consensus diagnoses. The results seem to indicate that digital image review of scanned slides is adequate for obtaining an accurate diagnosis for most types of lesions. These reviews suggest that digital images may be a useful and productive component in the PWG process. However, for complex and subtle lesions there may be limitations regarding diagnostic accuracy. In conclusion, the use of this technology in the PWG or peer review processes should be done with an understanding of such limitations.

Poster Abstracts

P11: The Diagnostic Accuracy of Digital Histopathology: Experiences at the NTP and NCTR

David E. Malarkey², Susan A. Elmore², Gabrielle A. Willson¹, Michael Boyle², Torrie A. Crabbs¹, E. Terence Adams¹, Jerry F. Hardisty¹, Greg R. Olson³, William M. Witt³, and Rodney A. Miller¹

¹Experimental Pathology Laboratories, Inc., RTP, NC

²National Institute of Environmental Health Sciences, RTP, NC

³Toxicologic Pathology Associates, Jefferson, AR, USA

The rapid technical advances in quality digital scanning of glass microscope slides, image processing and digital storage, combined with high-speed networking and improved personal computers, is making remote evaluation of digitally imaged tissue sections an increasingly viable option in histopathology evaluation. It is imperative, however, that this technology should not be automatically implemented in toxicologic pathology without due considerations of the accuracy, advantages, and disadvantages. Therefore, the National Toxicology Program (NTP) and the National Center for Toxicologic Research (NCTR) have evaluated the use of digital image microscopy in peer review and pathology working group (PWG) settings. The reviews were conducted in the PWG stage of eight (8) contemporary mouse or rat NTP and NCTR studies. In total over 25 pathologists were involved in the reviews comparing glass slide diagnoses to corresponding digital images of the same tissue. The conclusions of all eight PWGs were the same whether peer review was by light microscopy or digital microscopy. Overall there was a high concordance (86%; 500/579 slides in agreement, range 75-100%, n = 8 studies) of final diagnoses between the PWGs conducted with glass slides compared to digitally scanned slides. In summary, it appears the use of a digital image review can accomplish the objectives of a glass slide review. Digital image evaluation can enhance the PWG process either as a complement to the microscopic slide examination or eventually may become an accepted substitute for direct light microscopic examination of slides. Digital histopathology images are generally well-suited for the conduct of PWGs or interim pathology evaluations, seeking an expert opinion, teaching and publication purposes.

Poster Abstracts

P12: Quantitative Digital Pathology in Preclinical Safety Studies

Johannes Zimmermann, Florian Leiss, Kai Hartmann, and Martin Baatz
Definiens AG, München, Germany

High-throughput toxicology studies are heavily benefiting from recent advances in standardization of staining conditions and the automated acquisition of whole virtual slides. However, the manual evaluation of compound effects, being widely practiced so far, is extremely time consuming. Moreover, whereas human perception is excellent in the qualitative assessment of complex structures, it is limited in the quantitative description of biological phenomena. Due to the large experience required in histological judgments, inter-observer variability is another serious challenge. Hence, the fully automated generation of quantitative, non-equivocal readouts is crucial to resolve critical productivity issues for saving time and resources in preclinical studies.

A comprehensive and integrated environment for the automated analysis of images and data, Definiens, can be applied for a wide variety of preclinical safety studies. The system reliably detects regions of interest and quantifies within them staining intensities and morphological features of cells and cellular substructures. Spatial relationships and complex morphologies are described in detail by a high number of parameters, enabling an unprecedented richness of the readouts. The range of supported methodologies comprises brightfield as well as fluorescence, immunostains as well as in situ hybridizations. Tightly integrated is the mining of generated data for reporting purposes, quality control or correlation between different studies. In a large series of studies, Definiens image and data analysis has shown to significantly reduce study time by factor 3-5, to increase scoring efficiency, accuracy and objectivity and to relieve the pathologist from tedious routine tasks

Poster Abstracts

P13: Automated Digital Morphometry of Thyroid Gland Follicles Using Standard H&E-Stained Tissue Sections and Comparison to Manual Morphometry

Thomas J. Rosol^{1,2}, David R. Sweet¹, Joshia B. Pelot¹, and Mark E. Plaskow².

¹Department of Veterinary Biosciences, The Ohio State University

²American Health Technology, Inc., Columbus, Ohio, USA.

Introduction: Measurement of morphometric thyroid follicle parameters can be very helpful in the assessment of preclinical toxicology and endocrine disrupter studies. Unfortunately, manual morphometry of thyroid follicles is labor intensive and rarely performed. In addition, thyroid follicle size, colloid area, and follicular epithelial height are variable in single thyroid glands and in glands among experimental groups of animals. Pathological assessment of changes in thyroid follicles is qualitative and can vary between pathologists. Automated quantitative analysis of digital images of thyroid follicles can be used to improve the assessment of thyroid follicle morphology by increasing the sensitivity of identification of changes and standardizing the interpretation between pathologists and laboratories.

Methods: Thyroid follicular epithelium height, follicle area, colloid area, and follicular epithelial area of H&E sections of rat and mouse thyroid glands were measured manually using a Bioquant Osteo (ver. 11) image analysis system (100-300 per gland). A computer algorithm was developed that measured follicle area, colloid area, and follicular epithelial height automatically using standard digital images (jpeg, tiff) of H&E-stained thyroid glands. The data were tabulated using a web-based portal and database that enables secure image visualization and data viewing via the internet. The manual and automated data were compared for individual follicles and the population of follicles in a gland.

Results: Individual follicle measurements required 3 minutes per follicle (15 hours per 300 follicles). The automated algorithm required 30 sec. to measure 300 follicles. Colloid area of follicles was measured with a high degree of accuracy by the automated method (>98% accuracy compared to manual measurements). The thyroid follicle area and follicular epithelial height measurements were >88% accurate compared to manual measurements.

Conclusions: An algorithm has been developed that automatically measures colloid area, follicular area, and follicular epithelial height on digital images of H&E-stained thyroid glands. The algorithm was validated by comparison to manual measurements. It is critical to compare new automated techniques to a manual 'gold standard' method. The automated algorithm can work with images of the thyroid gland from any species. The alpha version of the algorithm performed very well when compared to manual morphometry. Correlation between colloid areas was 0.99 and follicular epithelial heights was 0.88. Time savings were dramatic between the automated and manual methods. Analysis can be gated by follicle or colloid area. Data and images are stored in a web-enabled, secure database that enables evaluation of data and viewing of images world-wide. Analyses can be performed on jpg, tiff, or other image formats.

Discussion: This automated tool is an important advance for quantitative morphometry and pathology of the thyroid gland since it only requires standard digital images of thyroid glands with H&E staining. The algorithm can be run in-house with secure web-based viewing of tabular and image data, and no slide transfer is required.

Poster Abstracts

P14: An Experimental Dog Model of Prostate Cancer for In Vivo Imaging and Therapeutic Research

Jill M. Keller¹, George R. Schade¹, Kim Ives¹, Xu Cheng¹, Thomas J. Rosol², Morand Pier³, Javed Siddiqui⁴, William W. Roberts¹, Evan T. Keller¹

¹Department of Urology, University of Michigan, Ann Arbor, MI

²Department of Veterinary Biosciences, The Ohio State University, Columbus, Ohio

³Department of Radiology

⁴Center for Translational Pathology, University of Michigan, Ann Arbor, Michigan, USA.

Prostate cancer is the most frequently diagnosed malignancy and the third leading cause of cancer-related death in men in developed countries. Animal models of prostate cancer, including a wide variety of transgenic, knockout, and xenograft mouse models, have proven extremely valuable in expanding our knowledge of this disease. However, prostate cancer is a complex, multifactorial disease process, and despite the advances provided by animal models, no existing animal model fully recapitulates all features of human prostate cancer. Besides humans, the dog is the only other large mammal that commonly develops spontaneous prostate cancer. Similar to humans, dogs develop benign prostatic hyperplasia (BPH), prostatic intraepithelial neoplasia (PIN), and invasive prostate cancer spontaneously with age. The dog prostate also shares a variety of anatomic and functional similarities with humans. In addition, the disease process in dogs with spontaneous prostate cancer is very similar to that of humans, including a tendency to metastasize to bone, a feature which mouse models of induced prostate cancer do not share. We developed a dog model of prostate cancer that would more fully represent the features of human prostate cancer than existing rodent models. Such a dog model will be invaluable in pre-clinical studies of diagnostic and therapeutic regimens for both human and canine prostate cancer.

Adult male 5-6-year-old beagle dogs (n=12) were immunosuppressed with cyclosporine A (12-40 mg/kg/day) and implanted with Ace-1 canine prostate cancer cells (3-6 x 10⁷ cells) using ultrasound-guided biopsy techniques. The Ace-1 cell line (developed by the Rosol lab) is an immortalized canine prostate cancer line from a spontaneous prostate cancer that forms osteoblastic bone metastases in nude mice after intracardiac injection (LeRoy et al., *Prostate*, 66: 1213-22, 2006; Thudi et al., *Prostate*, 68:1116-25, 2008; Thudi et al., *Prostate*, 71:615-25, 2011). Ace-1 tumors grew in all dogs in the prostatic capsule and parenchyma until euthanasia at 4-6 weeks and prostate volume increased from 15 to 22 cc³. The Ace-1 xenografts formed carcinomas that invaded the prostatic parenchyma, capsule, and vessels. Gross metastases occurred in the lungs and regional lymph nodes in 50% of the dogs.

Positron emission tomography (PET) scanning was performed in two dogs. The first dog was imaged using 5 mCi of ¹¹C-choline for 60 minutes and 5 mCi of ¹⁸F-choline for 60 minutes. No tumor tissue was identified. The second dog was imaged using 5 mCi ¹¹C-methionine and 5 mCi ¹⁸F-fluorodeoxyglucose (FDG). The PET scan using ¹¹C-methionine detected the primary tumor in the prostate gland and distant metastatic sites, include bone metastases.

The dog model of prostate cancer has great potential for advancing studies in this field. Potential areas of investigation include chemotherapy, imaging using radioactive tracers for novel molecular targets, surgical therapy, local ablative therapy, studies on growth, local invasion, and metastasis, bone metastasis studies on bone formation and resorption, and investigations on tumor microenvironment in a host that naturally develops prostate cancer.

Poster Abstracts

P15: Immunohistochemical characterization of proliferative lung lesions in SP-C/c-raf-1-BxB mice

Susanne Rittinghausen¹, Johanna Teloh^{1,2}, Dirk Schaudien¹, and Roman Halter¹

¹Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany

²University of Duisburg/Essen, Germany

The SP-C/c-raf-1-BxB mouse is used as a transgenic mouse model to study lung cancer. There are various reports available about histopathology of the lungs and molecular analysis of tissue, but only sparse data about immunohistochemical characterization of the proliferating cells.

For our recent investigations, lungs of SP-C/c-raf-1-BxB mice aged 6 to 13 months with macroscopic findings suggesting pulmonary tumor growth were dissected, fixed in 10% neutral buffered formaldehyde, and embedded in paraffin.

For immunohistochemical characterization of lung lesions, a panel of the following antibodies was applied to serial sections: surfactant protein C (SP-C), surfactant protein A (SP-A), secretory Clara cell protein (CC10), pan-cytokeratin (pan-CK, mixture of CK1, CK2, CK5, CK6, and CK18), cytokeratin 18 (CK18), thyroid transcription factor (TTF-1), Wilms' tumor protein (WT1), F4/80, galectin-3 (MAC-2), CD3, vimentin, CD31, PCNA, and Ki-67. Histo Red was used as chromogen.

Proliferating cells in the lung parenchyma - hyperplasia and neoplasia - were positive for SP-C, pan-CK, CK18, TTF-1, PCNA, and Ki-67, and negative for WT1, CC10, and SP-A. Immunohistochemical investigations demonstrated the type-II-cell nature of the lung tumors. In addition, they showed that in lungs with advanced stages of cell proliferation the type-II cells were surrounded by F4/80-positive macrophages and interspersed with large numbers of MAC-2-positive macrophages.

Immunohistochemical analysis of lungs with multiple lesions may enhance the tissue selection for molecular analysis by classifying cell proliferations and accumulations into predefined compartments.

Poster Abstracts

P16: Histopathology confirmed the specific uptake of DFMT (O-[18F] fluoromethyl D-tyrosine) in tumor metastasis but not in inflammatory infiltrates

Anna-Lena Frisk¹, Keith Graham¹, Anne Strube¹, Hideo Tsukada², Volker Gekeler¹, Sabine Zitzmann-Kolbe¹

¹Global Drug Discovery, Bayer Pharma AG, Berlin, Germany

²Hamamatsu Photonics H.H. Hamamatsu, Japan

Objective: The detection of tumors and localization of metastatic lesions and their differentiation from therapy induced inflammation is still a difficult obstacle for diagnosis and subsequent treatment decisions, thus contributing to an optimized cancer patient management and therapy control. At present [18F]-fluorodeoxyglucose (FDG) has been shown to have limited specificity due to its accumulation in inflammatory cells which have an increased glucose metabolism. To overcome this limited specificity of FDG, different amino acids have been investigated, one amino acid which shows great potential is DFMT (O-[18F]fluoromethyl D-tyrosine). DFMT has shown good uptake into HeLa tumors in nude mice with no accumulation in sterile induced inflammation sites and has been shown to image bone metastasis when human 786-O/luciferase renal cell adenocarcinoma cells were injected intracardially. The aim of this study was to investigate DFMT in an additional metastasis model for its capability to detect the primary tumor as well as metastatic lesions and differentiate metastatic from inflammatory sites. At the end of the study the results were confirmed by histopathology.

Methods: 4T1 mouse mammary carcinoma cells were implanted subcutaneously in female NMRI mice resulting in the formation of several lung metastases. The primary tumor was surgically removed on day 26 to allow more time for metastases growth in the lung. The radiosynthesis of DFMT was carried out using an indirect labeling method via the [18F] fluoromethyl bromide synthon. Micro-PET imaging was performed after removal of the primary tumor (day 28/29). After micro-PET imaging samples of tumor, scar and lung tissues were collected for autoradiography and histology.

Results: DFMT clearly visualized several metastases in the lung with no uptake in the lung parenchyma, which correlates with the histopathological findings in the lung. DFMT and FDG showed strong uptake at the site of the removed primary tumor with a stronger signal and broader signal with FDG when compared to DFMT. At histopathology the stronger signal of FDG was attributed primarily to inflammatory infiltrates and less to the presence of relapse of tumor growth. Whereas the weak and focused signal of DFMT could be histologically correlated to tumor tissue. The observed edema and inflammation was not depicted by PET DFMT imaging.

Conclusion: In this study histopathology proved to be a valuable tool to visualize the cellular morphology at the accumulation site of DFMT and FDG. Histopathology allowed a very specific morphological identification of the accumulation site of DFMT and FDG. There by it was possible to identify and specify that tumor tissue as well as inflammation was responsible for the FDG uptake, whereas DFMT was solely taken up by tumor tissue. DFMT is a promising, highly selective diagnostic PET tracer and these data qualify DFMT as a new potential imaging tracer for the in vivo detection of primary and relapsed tumors as well as metastases with high specificity to reliably differentiate tumor lesions from inflammation.

Poster Abstracts

P17: Subcutaneous injection sites: is the quality of the diagnosis improved when more than one tissue section is examined?

*C. Thuilliez, C. Clément and M.F. Perron-Lepage
Ricerca Biosciences SAS. Lyon, France*

At our Laboratory, the microscopic examination of subcutaneous injection sites routinely involves the evaluation of three longitudinal sections per tissue sample. Although this is intended to maximise the possibility of the section including the actual site of deposition of the injected material, it is time-consuming. We conducted a retrospective study to evaluate the relevance of the diagnosis in subcutaneous injection sites if only one section is examined.

Two rat studies (160 animals and 400 injection sites) and three primate studies (120 animals and 499 injection sites) were reviewed. All animals received saline or test item injected subcutaneously in the dorsum. Each site received 1 to 4 injections over the course of the study. In the rat studies, 3 or 4 sites were used and in primates, 4 or 10 sites. Each site was identified in-life by ink marks (a dot for rodents and a square for primates). The injection was given on the dot (rat) or in the centre of the square (primate). At necropsy, 3 adjacent pieces of skin, each 3 mm wide, were trimmed from each site. Piece 1 was in the middle, piece 2 lateral to it and piece 3 medial to it. The original study pathologists formulated overall diagnoses for each site, taken from evaluation of each of the 3 pieces.

One pathologist reviewed the three sections from each site with reference to the findings recorded by the original study pathologists and attributed each of the original diagnoses to the section(s) where it was observed. In each species, the studies and sexes were pooled but animals receiving saline or test item were evaluated separately. The percentage of findings in each section compared with the original overall diagnosis was calculated. The percentages were analysed statistically, comparing section 1 with the other two sections and with the original overall diagnosis.

In the rat, section 1 had a statistically significantly higher number of findings when compared to other sections, in control and treated animals. In the primate, section 1 did not differ from the other sections in control and treated animals. In both species, however, section 1 had a statistically significantly lower number of findings when compared with the original overall diagnosis.

In a previous study concerning the intramuscular injection sites, we demonstrated that examination of one section is sufficient to obtain a representative diagnosis. Contrary to this result, this retrospective study reveals that examination of three sections of subcutaneous injection sites improves the quality of diagnosis. For microscopic changes induced by subcutaneous injections, particularly in primates, one middle piece is not considered to be sufficiently representative of the whole site.

Poster Abstracts

P18: Set up a method for linear radiographic measurement of the longitudinal tibial axis in Sprague Dawley rats

Simone Borgonovo¹, Michele Ardizzone², and Mauro Di Giancamillo¹

¹Department of Veterinary Sciences for Health, Animal Production and Food Safety, Milan

²RBM S.p.A - Merck Serono, Colliere Jacosa (TO), Italy

The aim of this work was to set up a method for linear radiographic measurement of the longitudinal tibial axis in Sprague Dawley rats, to be used in a juvenile repeated-dose toxicity study, for determination of rat tibial length variations, in response to treatment with a GH-related compound, given at different doses.

The X-ray investigation was carried out on lateral view of the limb, excluding the craniocaudal view, due to the anatomic conformation of the shaft and the sample preparation methods (disarticulation) which did not allow the beam to be kept perpendicular with bone's long axis, generating variable geometric-projective distortions that hinder assessment.

Two different linear measurement methods were compared on 30 rats (males and females) right tibia, in order to find and standardize the more easily applicable and repeatable, precision being the same.

On the basis of the results obtained, comparing the measurements taken with the two different methods, it can be said that they are superimposable, since the difference between measurements taken with them on a single sample never exceeded 0.8 % of the values in males and 0.7% in females.

However, conditions being equal, one of the two methods was selected as candidate as more practical and easier to be repeated, compared to the other.

Poster Abstracts

P19: Serial Blood Sampling for Dried Blood Spot Technique in Toxicology Studies: Impact on Corticosteron, Histopathology and Clinical Pathology in Crl:CD (SD) Rats

*A. Lanzoni, I. Sartori, M. Sartori, E. Vicentini, S. Bertani, F. Crivellente, A. Criado, A. Casartelli, I. Faustinelli, L. Marocchio
Preclinical Biosciences, Aptuit, Verona, Italy*

In toxicity studies data are integrated with toxicokinetic (TK) evaluation to relate exposure with findings. In rodents, the TK profile is generally investigated on plasma obtained by serial blood sampling in dedicated animal from satellite groups. Dried Blood Spot (DBS) technique allows TK determination on limited amount of blood and it can be therefore applied on main study animals, with the advantage of decreasing the number of animals as well as allowing a robust correlation between toxicology findings and exposure.

This poster presents the impact and implications of DBS in a 7-day oral study on a standard rat model. Serial blood samples from male and female Crl:CD(SD) rats were collected at standard TK time points either automatically (by Accusampler® in cannulated animals) on Day 1 and 3 (group A); or manually (by tail venopuncture, VP) on Day 1 and Day 7 (group 2B) or on Day 7 only (group 3B). In a further group of animals (group 1B) only terminal blood collection was performed. Corticosterone was determined in groups A (to obtain physiological levels), 2B and 3B while clinical pathology and histopathology (adrenals, bone marrow, heart, kidneys, liver, lung, lymph node cervical and mesenteric, ovaries, spleen, stomach, testes, thymus) were assessed in 1B, 2B and 3B groups.

Higher corticosterone levels were observed only in VP females compared to cannulated animals. In these females, glucose increase was also noted. Minimal changes in haematological parameters were observed in VP groups, and were likely attributable to bleeding procedure and subsequent hematopoietic regenerative response. In particular, decreases in red blood cells parameters, associated with increase in reticulocyte populations were seen. Changes compatible with traumatic injury induced by the VP were observed at microscopic examination in the tail of some animals from both VP groups, consisting of acute, perivascular haemorrhage; this was associated with minimal fibrinogen increase noted seen in both sexes and in both groups.

These results indicate that serial blood sampling by VP for DBS technique application seems to increase corticosterone levels and blood glucose levels, with females more affected than males. Furthermore, it induces minimal variations in haematological and coagulation parameters, compatible with the repeated bleeding and traumatic injury induced by the procedure as confirmed by histopathological examination.

Although further investigations are required, we preliminary suggest that serial blood sampling by VP in rats for DBS technique application is well tolerated in normal rats used in toxicological studies.

Poster Abstracts

P20: Simulated Tail Venipuncture Transiently Increases Serum Cardiac Troponin I

Igor Mikaelian¹, Micaela Reddy¹, Gerry Hirkaler¹, Barbara Lenz¹, Lucette Doessegger², Thomas Singer²,
Mudher A. Albassam¹, Michael E. Dunn¹
Hoffmann-La Roche, Inc., ¹Nutley, NJ, USA and ²Basel, Switzerland

The laboratory procedures associated with compound administration and blood sampling may interfere with clinical pathology read-outs in rodent toxicology studies. The purpose of this study was to assess the effects of common animal handling procedures on serum cardiac troponin I (cTnI) which is a cardinal clinical biomarkers of acute myocardial damage. Serum cTnI concentrations were measured with the ultrasensitive Erenna™ method in femoral-catheterized male Crl:WI(Han) rats left undisturbed in their cage (UC); submitted to 5 min of isoflurane anesthesia (A); or placed into a rodent restrainer with submersion of the tails into a warm water bath for 2 min followed by simulated intravenous dosing in the tail vein (RR procedure). Mean baseline serum cTnI was 4.2 ± 4.3 pg/mL (mean \pm SD), with a range of 1.0-23 pg/mL and remained stable throughout the duration of the study. Serum cTnI concentrations 30 min and 2 hours after the RR procedure were increased to the 10.2-210 pg/mL range in most rats in the absence of histomorphologic findings. There were no changes in serum cTnI concentrations in rats submitted to the UC and A procedures. In a follow-up study, serum cTnI concentrations were in the range of 14.9 to 24.7 pg/mL in 3 of 16 rats 1 hour after the RR procedure. In this follow-up study, mean baseline was 2.6 ± 1.5 pg/mL, with a range of 0.6-7.4 pg/mL. These findings identify effects of the RR procedure on serum cTnI concentrations in rats. Also, anesthesia up to 24 hours after this procedure does not affect serum cTnI concentrations. Therefore, anesthesia is suitable for the assessment of this parameter as outlined in the recent context of use document recently issued by the United States Food and Drug Administration.

Poster Abstracts

P21: Could miRNA-126 become a translational biomarker of vascular injury?

Igor Mikaelian¹, Valeria Nicolini², Natalie D. Keirstead¹, Rosario Garrido¹, Barbara Lenz³, Christian Klein², Thomas Weiser³, Mudher A. Albassam¹, Thomas Singer³, Cristina Bertinetti-Lapatki³
Hoffmann-La Roche, Inc.,

¹Nutley, NJ, USA;

²Schlieren, Switzerland, and

³Basel, Switzerland

MicroRNAs (miRNAs) are 19-23 nucleotide-long non-coding ribonucleic acid (RNA) molecules. The high degree of conservation of miRNAs across species is the rationale for investigating miRNAs as potential translational biomarkers of tissue-specific toxicity, especially for those tissues and cells for which there is currently no specific and sensitive biomarkers such as the vascular beds. miR-126 is specifically expressed in endothelial cells (ECs) where it governs vascular integrity and angiogenesis. In man, its tissue expression is increased in multiple diseases associated with endothelial damage. Therefore we hypothesized that miR-126 was dysregulated in animal models of vascular injury (VI), and specifically in the mouse model of IL2-induced VI. The expression of miR-126 in normal mouse tissues based on locked nucleic acid *in situ* hybridization was restricted to ECs where it was strong in the nucleus and moderate in the cytoplasm of most cells. Mice administered IL2 had a strong positive signal in the plasma, which supports further investigating miR-126 as a circulating biomarker of VI. The expression of miR-126 in ECs of mice administered IL2 was often lost in the nucleus and increased in the cytoplasm, which supports a role of miR-126 in endothelial maturation. Finally, as the number of miR-126-positive ECs decreased, weak staining for miR-126 was detected in other cell types. This last observation likely identified transfer of miR-126 from ECs to non-ECs rather than *de novo* synthesis of miR-126 by non-ECs.

Poster Abstracts

P22: Adefovir- and Tenofovir-related renal changes from 28-day oral investigation on Sprague-Dawley rat: histopathology, electronic microscopy, genomics and urinary kidney biomarkers end-points

A. Piaia, Z. Dincer, E. Persohn, E. Tritto, D. Ledieu, V. Dubost, A. Mahl, U. Junker Walker, F. Pognan, P. Moulin, B. Kittel, and M. Uteng
Novartis Pharma AG, Basel, Switzerland

INTRODUCTION: Nucleoside reverse transcriptase inhibitors (NRTIs) are the basis of clinically successful anti-retroviral therapy to control HIV-1 infections. Despite this distinct benefit, NTRI-based therapies may have limitations due to potential organ toxicity such as kidney toxicity. Adefovir (ADF) and Tenofovir disoproxil fumarate (TDF) are two related NTRI drugs; ADF is no longer used for HIV-1 infection because of the high incidence of renal toxicity, while TDF has been occasionally linked to cases of proximal tubular dysfunction, Fanconi syndrome and acute tubular injury.

AIM: ADF and TDF were tested in an oral study, run on Sprague-Dawley rats in our facilities, to compare the nephrotoxic potential of the two compounds in a rodent model.

M&M: Doses (as base) were 11 and 28 mg/kg/day for ADF and 300 and 600 or 1000 mg/kg/day for TDF (each dose selected according to ~5 or ~20X human exposure). Renal function was assessed by a panel of urinary kidney biomarkers; renal lesions were characterized at histopathology, electron microscopy (EM) and gene expression profiling, after 28 days of administration.

RESULTS: 1. ADF dosed at 28 mg/kg/day resulted in nephrotoxicity, characterized microscopically by diffuse cortical tubular degeneration/regeneration with karyomegaly, mainly affecting proximal convoluted tubules (PCTs). Electron microscopy (EM) showed the presence of large cytolysosomes (autophagic vacuoles) filled with mitochondria at different stages of degradation, loss of cytoplasmic mitochondria and pronounced alteration in the appearance and distribution of other cellular organelles. Genomic investigation revealed up-regulation of classical kidney toxicity markers, stress-response induced genes, proteasome-associated genes and down-modulation of tubule-associated genes. Urine analysis revealed slight to marked increases in urine creatinine-normalized concentrations of numerous biomarkers for tubular injury (KIM-1, neutrophil gelatinase-associated lipocalin, osteopontin/SPP1, beta-2 microglobulin, calbindin) and moderate increase in urine total protein. At 11 mg/kg/day, nuclear enlargement of tubular epithelium and mild up-regulation of cell division-associated genes were seen.

2. TDF dosed at 1000 or 600 mg/kg/day was not tolerated, due to severe gastrointestinal toxicity observed at Day 6 or 7. TDF at 300 mg/kg/day resulted in increased tubular hyaline droplets (Chromotrope 2R positive and confirmed to be composed of α 2-microglobulin at immunohistochemistry) in the PCTs. As observed with ADF, tubular-cell nuclear enlargement was also observed with TDF treatment. At EM, hyaline-droplets accumulation correlated with increased number of secondary lysosomes (polygonal to irregular in shape). No other changes in cellular organelles were observed. In genomics and urine analysis, there were no toxicologically relevant changes.

CONCLUSION: The two tested NRTI drugs ADF and TDF revealed two different toxicity profiles in Sprague-Dawley rats in this study. Treatment with ADF caused dose-dependent nephrotoxic effects mainly centered in the proximal convoluted tubules and suggested a mitochondrial-degeneration/depletion mechanism of toxicity. Treatment with TDF caused minor kidney effects (nuclear enlargement of the tubular epithelium and hyaline-droplet accumulation) at the 300 mg/kg/day. With higher doses, gastrointestinal toxicity limited further investigations on the kidney. The cause of the increased hyaline droplets with TDF was not clear in this study. Nuclear enlargement of the tubular epithelium was the only common finding observed in the kidney of ADF- and TDF-treated rats.

Poster Abstracts

P23: Identification of in vivo biomarkers for vancomycin-induced nephrotoxicity

Hang Sik Roh, Ji Hyeon Seok, Ji Young Yun, Ji Hyun Yun, Tae Sung Kim, Chi Won Song, Young Hee Jo, Jong Kwon Lee, Soon Young, Han and Ja Young Jeong
Toxicological Research Division, Toxicological Evaluation and Research Department, National Institute of Food and Drug Safety Evaluation, Korea

Vancomycin (VAN) is a glycopeptide antibiotic used to treat gram-positive infections. Nephrotoxicity is a common side effect observed with vancomycin therapy. Since nephrotoxicity affects the development of drug candidates, it is important to detect their toxicity at an early stage of drug development. In this study, we aimed to identify in vitro biomarkers for the vancomycin-induced nephrotoxicity which can replace the in vivo testing. Therefore we measured known urinary nephrotoxic biomarkers [albumin, kidney injury molecule-1(KIM-1), clusterin, β 2-microglobulin, cystatin-c, trefoil factor 3(TFF3)] in kidney in mice. Groups of ten male BALB/c mice were treated with 14 daily iv doses of vancomycin (50, 100, and 200 mg/kg) or saline, and sacrificed on day 15 and 17. Clinical chemistry and histopathology demonstrated kidney injury. RNA and protein were extracted from the kidney, and RT-PCR and western blot were performed to evaluate expression profiles of the selected biomarkers. Body weight and absolute kidney weight of the high dose group were significantly decreased. In a histopathological study, we observed tubular necrosis in treated mid dose and high dose groups. Among those biomarkers displaying the changes in vancomycin-treated mice were KIM-1 and clusterin. Our results further substantiate the use of gene markers of kidney toxicity such KIM-1 and clusterin as indicators of renal injury.

Poster Abstracts

P24: Intermittent Co-administration of a Gamma Secretase Inhibitor with Dexamethasone Mitigates Intestinal Goblet Cell Hyperplasia in Rats

Shirley A. Aguirre, Ling Liu, Natilie A. Hosea, Leigh Ann Burns-Naas, Sophia Randolph, Robert H. Denlinger, and Bora Han
Pfizer Inc., San Diego, CA, USA

Perturbation of intracellular Notch signaling has been implicated in oncogenesis and this signaling pathway is a target of interest in cancer therapy. Gamma (γ) secretase plays a key role in Notch-dependent nuclear signaling however, as expected, inhibition of γ secretase by a potent inhibitor (GSI), PF-03084014, resulted in gastrointestinal goblet cell hyperplasia (GCH). Development of GCH was associated with the inhibition γ secretase and Notch receptors signaling pathways on enterocytes and favored a predominance of mucous secreting cells over presence of normal absorptive enterocytes on intestinal villi. Potential mitigating effect of the intestinal GCH was assessed in Sprague-Dawley (SD) rats when co-treated with dexamethasone in either a pre-treatment or concurrent intermittent dosing regimen using a one week on and one week off dosing schedule for 28 days in a repeat dose studies. Results showed intermittent combination dosing with a clinically relevant oral dexamethasone dose of 1 mg/kg given on weeks 1 and 3 with daily dosing of GSI at 100 mg/kg/day for 1 month blocked GCH in the small intestines and reduced the incidence of GCH in the large intestines. In the same study, pre-treatment with dexamethasone one week before treatment with 100 mg/kg/day GSI was insufficient to block development of GCH in the intestines. The data presented here show, in principle that, co-administration of dexamethasone in an intermittent regimen minimized the gastrointestinal side-effects associated with continuous dosing of a chemotherapeutic agent (PF-03084014) and enables continuous dosing of this chemotherapeutic agent.

Poster Abstracts

P25: Lack of Oral Toxicity in 90-Day Study of the Food Additive, Gum Ghatti

Robert Maronpot¹, Jeffrey Davis², Glenda Moser², Susan Borghoff², Dipak Giri², and Shimmo Hayashi³

¹Maronpot Consulting LLC, Raleigh, NC

²Integrated Laboratory Systems, Inc., Research Triangle Park

³San-Ei Gen F.F.I. Inc., Osaka, Japan

Gum ghatti is a polysaccharides of natural origin used in foods as thickening, gelling, emulsifying and stabilizing agent. For evaluation, groups of 10 male and female Sprague Dawley rats were exposed to 0, 0.5, 1.5, and 5.0% of gum ghatti in AIN-93M diet for 90 days in accordance with OECD GLP guidelines. All animals survived to terminal necropsy and there were no significant in-life, body weight, feed consumption, ophthalmological or neurological findings. There were no treatment-related clinical pathology changes or gross lesions. The only tissue weight change was an expected increase in cecal weight with and without contents in the 5% groups. In H&E-stained tissues there was equivocal minimal to mild cecal crypt hyperplasia and elongation in 6/10 high dose male rats and moderate focal ulceration with inflammation in 2/10 high dose females. To confirm potential gum ghatti-related colonic lesions, a second 90-day study was conducted in groups of 20 female rats exposed to 0 or 5.0% gum ghatti in AIN-93M or NIH-07 diet. The only reproducible findings were increased cecal weights in rats exposed to 5.0% gum ghatti. There were no gum ghatti-related histopathological changes in the colon or cecum of animals fed either diet. One female control on the AIN-93M diet had a focal colon ulcer with inflammation. A PWG unanimously agreed that the focal colon ulcers with inflammation were sporadic changes not attributable to gum ghatti and concluded that neither cecal or colon changes posed a human health concern.

It is concluded that the dietary NOAEL level for gum ghatti in both studies is 5%.

Poster Abstracts

P26: Expression of epithelial cell adhesion molecule and proliferating cell nuclear antigen in diethylnitrosamine-induced hepatocarcinogenesis

Jin Seok Kang¹, Young-Il Park², Hwan-Goo Kang², Hyunjung Lee³, Kiho Park³, Yun-Seok Lee⁴, Soohee Kim⁵ and Doug-Young Ryu⁵

¹Department of Biomedical Laboratory Science, Namseoul University, 91 Daehak-ro, Seonghwan-eup, Sebuk-gu, Cheonan, 330-707

²Toxicology Laboratory, Toxicology and Residue Chemistry Division, Animal, Plant and Fisheries Quarantine and Inspection Agency, MIFAFF, 175 Anyang-ro, Manan-gu, Anyang, 480-757

³Laboratory of Genomics and Quantitative Real-Time PCR, Biomedical Research Institute, Seoul National University Hospital, 101 Daehakno Jongno-Gu, Seoul, 110-744

⁴Department of Health Administration, Namseoul University, 91 Daehak-ro, Seonghwan-eup, Sebuk-gu, Cheonan, 330-707

⁵College of Veterinary Medicine, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul, 151-742, Korea

To clarify the role of stem cells in hepatocarcinogenesis, epithelial cell adhesion molecule (EpCAM) and proliferating cell nuclear antigen (PCNA) expressions were investigated in mouse hepatic tumors and embryonic cell lineages. *In vivo*, ten ICR mice were treated with diethylnitrosamine (DEN) at 14 days of age, and were sacrificed at 36 weeks after DEN treatment to get the hepatic tumors. *In vitro*, mouse embryonic stem cells (ESCs), hepatic progenitor cells (HPCs) and hepatocyte-like cells (HCs) were treated with DEN at four doses (0, 1, 5 and 15 mM; G1, G2, G3 and G4, respectively) for 24 h on the differentiation stages of days 0, 22 and 40 and RNA was isolated. *In vivo*, seventy-one hepatic tumors were obtained from DEN-treated mice. EpCAM expression was increased mainly in hepatic tumor cells, even though it was also detected in surrounding normal-looking cells. Double staining showed that EpCAM and PCNA were co-localization in many tumor cells. *In vitro*, EpCAM expression was significantly different in G4 at day 0 ($p < 0.01$) and in G2, G3 and G4 at day 40 ($p < 0.01$) and PCNA expression was significantly different in G3 and G4 at day 0 ($p < 0.01$) and G2, G3 and G4 at day 22 ($p < 0.01$) and G2 at day 40 ($p < 0.01$) compared with that of G1 at each time point. Taken together, EpCAM expression was increased in DEN-induced tumors associated with PCNA and their expressions were altered by DEN treatment. It suggests that EpCAM may be modulated as the progeny of the adult liver stem cells differentiation toward hepatocytes but increased during DEN-induced hepatocarcinogenesis.

Poster Abstracts

P27: Evaluation of cardiotoxicity induced by peroxisome proliferator-activated receptor gamma agonists in mice

Hyun-Il Yang¹, Woo-Sik Kim², Dal-Hyun Kim² and Jin Seok Kang¹

¹Department of Biomedical Laboratory Science, Namseoul University, 21 Maeju-ri, Seonghwan-eup, Cheonan 330-707

²Division of Pharmacology & Toxicology, Hyo-Jong Research Institute, Chong Kun Dang, 464-3 Jung-dong, Giheung-gu, Yongin 446-527, Korea

It has been reported that heart toxicity is induced by peroxisome proliferator-activated receptor gamma (PPAR- γ) agonists in some patients or animal models for diabetes. CKD-501 is a new PPAR- γ agonist used for the management of non-insulin dependent diabetes mellitus (NIDDM). In this study, we investigated potential cardiotoxicity of CKD-501 and compared its toxicity with that of rosiglitazone or pioglitazone using db/db mice. After 12-week repeated administration of CKD-501 at doses of 3, 10 and 30 mg/kg/day or rosiglitazone at doses of 10 and 30 mg/kg/day or pioglitazone at doses of 200 and 540 mg/kg/day, animals were sacrificed for investigation of hearts. Diameters of left ventricles and cardiomyocytes in left ventricles of each group were measured. Lipid accumulation and apoptosis in heart muscle of each group were examined by oil red O staining and TUNEL staining, respectively. Diameters of left ventricles were significantly increased in high dose treatment of pioglitazone compared to control ($p < 0.05$), while other groups showed a tendency for an increase. All test chemicals induced significantly the increase of area of cardiomyocytes in heart compared to control ($p < 0.01$), in regular order as pioglitazone > rosiglitazone \geq CKD-501. However, lipid accumulation and apoptotic changes in heart were not observed in all dosing groups. Taken together, side effects of CDK-501 in heart are relatively lower than that of pioglitazone and similar to rosiglitazone. It is suggested that that CDK-501 may be safer compared to reference compounds in clinical use for NIDDM.

Poster Abstracts

P28: Comparative *in vivo* study of porous bioceramics for osteoconductive and osteoinductive response

Hye-Rim Lee¹, Han-Jun Kim¹, Ji-Seung Ko¹, Myun-Whan Ahn², Sukyoung Kim³, Sun Hee Do¹

¹College of Veterinary Medicine, Konkuk University, Seoul 143-701

²Department of Orthopedic surgery, College of Medicine, Yeungnam University, Daegu 705-717

³School of Materials Science and Engineering, Yeungnam University, Gyeongsan 712-749, Korea

Previously, we observed porous calcium phosphate ceramics, calcium metaphosphate (CMP), hydroxyapatite (HA), and collagen-grafted HA (HA-Col), had osteogenic potency by up-regulation of type I collagen, osteocalcin, ALP, Runx2, and RANKL in MC3T3-E1 cells and adipose tissue-derived stem cells (ADSCs). The present study evaluated the bone forming capacity of these bioceramics in rat models using femur defects and intramuscular implants. For evaluation of osteoconductive potency, cortical bone defect was created in the diaphysis of femur using dental drill, then each bioceramic including CMP, HA, and HA-Col was implanted to the lesion. Further osteoinductive potency *in vitro* with ADSC acquired from different properties, *in vivo* bone formation was evaluated using intramuscular implantation of bioceramics for osteogenic differentiation and /or exophytic bone formation. After 12 weeks of implantation, excised samples were harvested and used for hematoxylin and eosin (HE) staining and immunohistochemistry, and RNA/protein extraction from paraffin embedded tissues. Histological analysis showed that newly-formed stroma-rich tissues were observed in all the implanted regions. IHC for type I collagen revealed positive immunoreaction in the bone and muscles presented to all the implantations. In addition, ALP, a protein involved in bone metabolism, was also detected by IHC staining. Especially, intramuscular implanted region showed strong positive immunoreactivity, which further confirmed stimulation effects of each bioceramic on osteogenesis by mRNA expression and immunoblotting. These results indicate that smart bioceramics, CMP, HA, and HA-col, could induce osteoconduction and osteoinduction *in vivo* although mature bone formation including lacunae, osteocyte, and mineralization was not observed until 12 week after implantation.

Poster Abstracts

P29: Toxicity Studies of an Agrochemical Fungicide WP Formulation (Cymoxanil 6% and Propineb 70%) in Sprague- Dawley Rats

Nabil Hailat

University of Science and Technology, Department of Pathology and Public Health, Faculty of Veterinary Medicine, Irbid, Jordan

Toxicity studies of an agrichemical fungicide WP formulation (Cymoxanil 6% and Propineb 70%) in Sprague- Dawley rats were conducted. These were acute oral, subchronic oral and subchronic dermal toxicities. A total of thirty six rats were used in this study; twelve rats in each treatment were used divided into two groups (control and treated). The rats were observed for clinical signs of toxicities and behaviour during the period of experiment. It was found that in the acute oral toxicity; the Median Lethal Dose (LD₅₀) is more than 5000mg/kg body weight. The subchronic oral toxicity studies revealed no significant changes in the mean body weights, internal organs, blood parameters and liver function enzymes compared to the control group. In addition, the results of the subchronic dermal toxicity studies indicated similar findings; no significant differences were found when the means were compared with the control group. Gross and histopathological examinations of the internal organs (liver, kidneys, heart, spleen, skin and lungs) in the treated groups were conducted and there was no significant changes in these organs as a result of treatments. These three studies collectively suggested this formulation was slightly toxic to practically non toxic, and is classified in category 5 in the Global Harmonized System (GHS). The results will be presented in tables and will be discussed.

Poster Abstracts

P30: Vertebral osteomyelitis with spinal cord compression after subcutaneous injection of vaccine in a BALB/c mouse

*Yu-Ling Chen, Tzu-Yu Chen, Yi-Ying Chiu, Hsiu-Ling Liao and Kan-Hung Lee
National Laboratory Animal Center, National Applied Research Laboratories, Taiwan*

One 43-week-old BALB/c mouse was observed with weakness in hind limbs and could not be grasped by the hind limbs by the animal taker one month after administration of a vaccine by subcutaneous injection in an immunization study. One of 100 mice showed clinical signs after single injection. This animal was submitted for pathology examination. Clinical signs included emaciation, hunched back, posterior paresis, weakness with dragging but sometimes the movement of left hind limb was observed (not completely lost function). Neurologic examination of pain reflex was positive in both hind limbs. Macroscopically, there were several areas of hemorrhage and some yellow-white material accumulation in the subcutis of the upper back and one of them adhered to the skin. Histopathologically, pyogranulomatous inflammation from dermis to deep muscular layer and vertebrae observed. Thoracic and lumbar vertebral osteomyelitis with compression of the thoracic spinal cord following the severe pyogranulomatous inflammation resulted in paresis of both hind limbs.

Osteomyelitis is most commonly caused by the bacterium *Staphylococcus aureus*, but other bacteria can cause it too. These pathogens usually enter the body's tissues through an open wound. Deeper wounds that are left untreated can become infected, and lead to adjacent bone infection. However, contamination during injection process (incomplete disinfection or contaminated injection materials) is probably the most possible cause of these lesions.

Poster Abstracts

P31: Brain Lesions Associated with Vitamin E Deficiency in Muscovy ducks

Sontana Mimapan^{1*}, Phanom Saijit², Jadsada Ratthanophart³, Thanongsak Mamom⁴, Isarayos Sirikanoke³, Ladda Trongwongsa¹

¹Pathology Section

²Biochemistry section

³Epidemiology section National Institute of Animal Health, Kasetklang, Chatuchak, Bangkok 10900

⁴Department of Pathology, Faculty of Veterinary Medicine, Mahanakorn University of Technology, 140 Cheum Sampan Road, NongChok, Bangkok, 10530, Thailand.

Introduction: Vitamin E plays an important role as an anti-oxidant in human and animal body. However, the mechanism and function of this vitamin are unclear (Norman 1966). Furthermore, many studies have been shown that vitamin E deficiency can cause muscular dystrophy, exudative diathesis and encephalomalacia (Dam et al., 1950). In particular, day-old chickens have been studied to characterize the deficiency in alpha-tocopherol leading to encephalomalacia which is characterized by necrosis of neuron and gliosis. Unfortunately, few studies reported the effect of vitamin E deficiency in muscovy ducks. Here, we present the clinical signs and lesions in the brains of muscovy ducks suffering from vitamin E deficiency.

Materials and Methods: Case history: Five muscovy ducks (age about 5-6 months) with clinical signs including incoordination, ataxia, head backward and downward retraction from a farm in central Thailand were sent for investigation to the National Institute of Animal Health (NIAH). **Necropsy and histopathology:** All animals were euthanized after blood collection. Samples were obtained and routinely processed for histopathology. Simultaneously, those samples were sent to investigate bacteriological, fungal and virological infections as well as test for toxin in gizzard content, livers and kidneys. **Biochemistry:** 1. Blood from five-affected and two healthy muscovy ducks from this farm were collected to investigate vitamin E and minerals by atomic absorption spectroscopy 2. Food and water in the farm were sent for analysis of pesticide like organophosphate and carbamate contamination 3. Gizzard content, livers and kidneys were sent to investigate aflatoxin. **Virological and Bacterial examination:** Tissue samples were sent for viral isolation and bacterial culture.

Results: Microscopic observation reveals diffuse satellitosis characterized by glial cells proliferation around damaged neuronal cell bodies, demyelination and gliosis in cerebellum of all five-affected ducks. In two healthy ducks, the level of vitamin E in serum is 1.55 ug/mL (average), whereas in five-affected muscovy ducks the average levels of vitamin E is 0.66 ug/mL. Moreover, there is no organophosphate or carbamate found in liver and kidneys and also no pesticide contaminated in food and water. **Importantly,** No bacteria, virus and fungus were isolated from all specimens.

Discussion: From our study, we conclude that lesions we found in the brains of affected ducks might be caused by vitamin E deficiency. Unfortunately we do not have normal standard value of vitamin E in serum of ducks in Thailand, but in this study affected ducks showed diffuse satellitosis corresponding with the lower level of vitamin E in serum than the vitamin E level in serum of healthy ducks. It is possibly that brain lesions found during the stage of vitamin E deficiency in this case might result in loss of nervous coordination and control. Moreover, after adding vitamin E supplement in animal feed, there were no such clinical signs observed from Muscovy ducks in this farm.

Poster Abstracts

P32: Induction of gastric atrophy following orally administration of fumonisin B1 in mice

Alizadeh Ali Mohammad^a, MohammadGhasemi Fahimeh^a, Zendehtdel Kazema,
Kamyabi-moghaddam Zahra^b, Tavasoly Abbas^b, Amini-njafi Fatemeh^b, Khosravi Ali Reza^c
^aCancer Research Center, Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran
^bDepartment of Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Fumonisin B1 is a group of toxic and carcinogenic mycotoxins which basically contaminate the grains and their products. We aimed to study the atrophic effect of orally administered fumonisin B1 (FB1) on gastric mucosa in mice. Twenty-nine female mice were divided into the treatment (n=15) and control (n=14) groups. The treatment group received FB1 (150 mg/kg diet) for 16 weeks. The gastric atrophy was scored using grading criteria modeled on the updated Sydney System. Immunohistochemistry was performed using TUNEL, Bcl-2 and Bax staining to evaluate apoptotic changes. Mild to moderate gastric atrophy was seen in the gastric mucosa in treated animals ($p < 0.05$). Parietal cells significantly decreased in the treatment group in comparison with the control group ($p < 0.05$). The number of TUNEL- and Bax positive cells significantly increased in FB1 treated group compared to the control group ($p < 0.001$). We also found a significant decrease in Bcl-2 positive cell numbers and Bcl-2/Bax ratio in the treated animals compared to the controls ($p < 0.01$). Orally administered fumonisin B1 caused atrophy and apoptosis induction, Bcl-2 inhibition and Bax protein overexpression in mouse gastric mucosa. These findings indicate that FB1 poisoning can have toxicopathological effects such as gastric gland atrophy and apoptosis on mice gastric tissue.

Poster Abstracts

P33: Histopathological Study of Acute and Chronic Toxicity Induced by Azole Pesticides in *Artemia Urmiana*

Aliasghar Tehrani¹, Javad Javanbakh², Naghmeh Homayounfar³, Amirali Solati⁴, Mehdy Aghamohammad Hassan⁵, Zahra Kamyabi-moghaddam², Hamid Akbar^{4,3,6}

¹Department of Pathology, Faculty of Veterinary Medicine, University of Urmia, Urmia, Iran

^{2,5}Department of Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

⁴Department of Veterinary Medicine, Islamic Azad University, Saveh, Iran

⁶Department of Clinical Science, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

⁶Department of Clinical Science, Faculty of Veterinary Medicine, University of Urmia, Urmia

Artemia Urmiana (Artemide), the brine shrimp larva, is an invertebrate used in the alternative test to determine toxicity of chemical and natural products. In the present study, Diniconazole and Cyproconazole toxins were used at 0.01ppm _ 100ppm and 0.001_ 10ppm concentrations respectively. The larva mortality was assessed at 0.5h, 2h, 6h, 12h, 1day, 2days, 3days, 4days, 5days, 6days and 7days after incubation. The results demonstrated that:

- Cyproconazole (100ppm) and Diniconazole (≥ 10 ppm) are able to produce acute toxicity
- Both toxins were more effective on L₂ (In this study, some stages of *Artemia* life cycle selected which play important role in husbandry and nutrition of aquatic animals such as Napleus (L₁), first stage of Metanapleus (L₂), and adults).
- Adults were more resistant to both toxins
- Toxicity of Diniconazole was higher than Cyproconazole.

Histopathological analysis for the Diniconazole, group, revealed that it caused necrosis, cellular swelling, fibrinous excretions in alimentary canal, decrease of gametic cells in males and Nurse Cell necrosis in ovaries

Cyproconazole toxin treated *Artemia* presented lesions such as degeneration of compound eye conical cells, hypertrophy, cellular swelling, hyperplasia and severe necrosis in digestive tract epithelial cells. In addition both toxins are able to trigger hyaline degeneration and muscular necrosis in *Artemia*.

Poster Abstracts

P34: DMBA (7, 12-dimethylbenzanthracene) induces dose dependent multi-organ tumors in BALB/c mice

Krishna Duro de Oliveira¹, Marcelo Vannucci Tedardi², Gabriela Uliana Avanzo¹, Marcelo Monte Mór Rangel¹, Jose Luis Avanzo¹, Heidge Fukumasu², Kurapati Venkata Kesava Rao¹, Idercio Luiz Sinhorini², Maria Lúcia Zaidan Dagli¹

¹Department of Pathology, School of Veterinary Medicine and Animal Science;

²Department of Basic Sciences, School of Animal Science and Food Engineering, University of São Paulo, Brazil.

Polycyclic aromatic hydrocarbons are known carcinogens used in experimental models. In this study, the carcinogen DMBA (7,12-dimethylbenzanthracene), diluted in corn oil, was administered by gavage to BALB/c mice at weekly doses of 1 mg per animal for 1, 3, 6 or 9 weeks. Animals were weighed and monitored weekly until death. Remaining animals were euthanized at the age of 53 weeks. At necropsy, representative fragments of neoplasms were harvested and routinely processed for microscopy. Of the 70 mice treated with various doses of DMBA, 67.14% developed some sort of tumor, and 22 (31.43%) developed mammary tumors. Adenoacanthoma was the most commonly diagnosed histological type of mammary cancer. Lung (17.14%), lymphoid tissue (11.43%), stomach (7.14%) and skin (1.43%) were also primary sites of tumor development. One-third (33.33%) of the mice receiving 1 mg of DMBA developed lung cancer. The administration of DMBA was therefore shown to be an efficient model of carcinogenesis in mice, especially for the study of breast cancer, when using the highest dose, and lung, when using the lowest dose. These results allow us to better understand the model, which can be used in chemopreventive studies.