Book of abstracts

Summer School

GENOMIC MEDICINE

Bridging research and the clinic

3.–7. May 2016
Portorož, Slovenia

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GENOMIC MEDICINE –
Bridging research and the clinic
Summer school
Book of abstracts

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Advanced Regional Translation of Excellence into Medical Innovation for Delayed Aging

Center odličnosti za translacijsko medicino
SIC PARVIS MAGNA
Centre of Excellence for Translational Medicine

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WELCOME ADDRESS

Dear colleagues,

We are pleased to welcome you to the Summer school: GENOMIC MEDICINE – Bridging research and the clinic, taking place in Portorož, Slovenia, from May 3rd to May 7th 2016 at the Hotel Histrion.

The program covers a wide range of topics related to genomic medicine (also known as personalized medicine), pharmacogenomics and epigenomics and translation of basic research into clinical practice and public health. Important topics of interest are identification of predictive and prognostic biomarkers and novel molecular targets for tailored therapeutic interventions for diseases linked to oxidative stress and age-related diseases and disabilities. Rare diseases and rare cancers, especially epidemiology, pathogenesis/pathology, and treatment modalities for pancreas cancer will be among the topics of the school.

The Summer school is organized by Pharmacogenetics laboratory, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana as a collaborative action between Artemida Teaming project, the EUPancreas COST Action (BM1204), the EU-ROS COST Action (BM1203), SERBORDI-Sinn and CASyM and is endorsed and supported by U-PGx, The Golden Helix Institute of Biomedical Research, Genomic Medicine Alliance, ESPT and Eu-PIC.

Such a collaborative action will maximize interactions and encourage the exchange of knowledge and experience and possibly stimulate new collaborations that will facilitate the translation of novel findings into preventive, personalized curative, promotional and rehabilitative health care services to improve health outcomes, reduce health inequalities and to promote wellbeing and active and healthy ageing in Slovenia, the C&SE European region and wider area.

We hope that we have created a friendly and stimulating atmosphere that will enable the formation of new collaborations. We hope that you will enjoy both the science and the beautiful Slovenian coast during this summer school.

Prof. Dr. Vita Dolžan
Chair of the International Scientific Committee and Organizing committee

Assist. Dr. Katja Goričar
Co-chair of the Organizing Committee

COMMITTEES

International Scientific Committee
- Vita Dolžan, chair (University of Ljubljana, Slovenia)
- Henk-Jan Guchelaar (Leiden University Medical Center, The Netherlands)
- Magnus Ingelman-Sundberg (Karolinska Institutet, Sweden)
- Núria Malats (Spanish National Cancer Research Centre, Spain)
- George Patrinos (University of Patras, Greece)
- Sonja Pavlović (Institute of Molecular Genetics and Genetic Engineering University of Belgrade, Serbia)
- Ron van Schaik (Erasmus Medical Center, The Netherlands)

Organizing Committee
- Vita Dolžan, chair
- Katja Goričar, co-chair
- Barbara Jenko
- Irina Milisav Ribarič
- Peter Juvan

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GENERAL INFORMATION

Congress venue
The event will take place at the **Hotel Histrion, Obala 2, Portorož, Slovenia**.

Registration and information desk
Tuesday, 3. 5. 2016 8:30–16:00 in the lobby of the Hotel Histrion, Portorož.
Wednesday, 4. 5. 2016 8:00–10:00 in the lobby of the Hotel Histrion, Portorož.
Thursday, 5. 5. 2016 8:00–10:00 in the lobby of the Hotel Histrion, Portorož.
Friday, 6. 5. 2016 8:00–10:00 in the lobby of the Hotel Histrion, Portorož.
Saturday, 7. 5. 2016 8:00–10:00 in the lobby of the Hotel Histrion, Portorož.

The certificate of attendance will be issued at the registration desk.

Name badges
All participants will receive name badges upon registration and are kindly requested to wear badges during all sessions and events of the summer school.

Credits
All participants that wish to receive ECTS or CME credits have to sign the appropriate attendance list every day.

Presentation preview and deposition
Presentation preview point where speakers can check and load their presentations will be available in the lecture hall. Speakers are kindly requested to bring their presentations in the lecture hall where the talk will be given during breaks before sessions.

Poster display area
Poster session will be held in the lecture hall of the Hotel Histrion.

Presenters are kindly asked to mount their posters on Tuesday, 3. 5. 2016, and remove them on Saturday, 7. 5. 2016.

Presenters are responsible for setting and removing the posters. Material for mounting the posters will be available at the venue. Authors are kindly requested to be present at their poster board for the duration of the poster session.

Internet access
Internet access will be available during the summer school.

Coffee breaks and lunches
Coffee breaks will be arranged in the lobby in front of the lecture hall of the Hotel Histrion. Lunches will be arranged in Restaurant Pristan of the Hotel Histrion and are included in half-board packages. For those attending the summer school for one day, vouchers will be available at registration desk. Otherwise, lunch vouchers are available at the reception of Hotel Histrion. All dinners are included in the registration fee and will be held in restaurants as scheduled.

Currency and banking
Slovenian official currency is Euro.

Important phone numbers and emergency calls
For any additional information during the summer school, or in case of emergency, please call:
Barbara Jenko: **+386 31 283739**
Emergency number: **112**
Police: **113**

Social activities for all registered participants

Tuesday, 3. 5. 2016
19:30 Welcome reception Hotel Histrion

Thursday, 5. 5. 2016
16.30 Excursion to Piran
19.30 Dinner in Piran

Friday, 6. 5. 2016
19.30 Gala dinner Hotel Histrion

PROGRAMME OUTLINE

	Tuesday 3. May	Wednesday 4. May	Thursday 5. May	Friday 6. May	Saturday 7. May
Morning session	Satellite Workshop: In vitro and in vivo models for development of novel diagnostic and therapeutic approaches	Pharmacog-enomics and pharmacoepig-enomics	Oxidative stress and disease Gene-environment interactions	Bioinformatics and biostatistics for translational research Emerging technologies in pharmacog-enomics	Translation to clinical practice Closing plenary lecture
Afternoon session	Opening plenary lecture NGS in genomic medicine and rare diseases Welcome reception	Pharmaco-genetics and personalized medicine Dinner	The needs and directions for research and development services in pharmacy and biomedicine: round table discussion Excursion to Piran Dinner in Piran	Cancer genomics New approaches to personalized cancer treatment Gala dinner	

DETAILED PROGRAMME

DAY 1 TUESDAY, 3. MAY 8.30-19.30

	Programme
	Satellite Workshop: In vitro and in vivo models for development of novel diagnostic and therapeutic approaches **Chairpersons: Antonio Cuadrado, Tamara Lah Turnšek**
8.30—9.00	Registration
9.00—9.30	**Delilah F.G. Hendriks**, Karolinska Institute, Sweden Novel in vivo like in vitro spheroid systems for prediction of drug response, long term adverse reactions and for studies of liver disease
9.30—10.00	**Metka Filipič**, National Institute of Biology, Slovenia HepG2CDKN1A–DsRed biosensor system for rapid and simple detection of genotoxic agents
10.00—10.30	**Tamara Lah Turnšek**, National Institute of Biology, Slovenia Experimental oncology from in vitro to in vivo: Zebra fish model
10.30—11.00	Coffee break
11.00—11.30	**Sergej Pirkmajer**, Faculty of Medicine, University of Ljubljana, Slovenia Skeletal muscle cells as a model for identification of novel targets for insulin resistance/type 2 diabetes treatment: to starve or not to starve?
11.30—12.00	**Antonio Cuadrado**, Autonomous University of Madrid, Spain Models of neurodegenerative diseases
12.00—12.30	Discussion
12.30—14.00	Lunch break, Registration

	Summer school programme
14.00	Opening address
	Opening plenary lecture
14.30—15.30	**Ron van Schaik**, Erasmus MC, Rotterdam, The Netherlands Do you have your DNA passport?
15.30—16.00	Coffee break
	NGS in genomic medicine and rare diseases **Chairpersons: Maja Stojiljkovic, Sonja Pavlovic**
16.00—16.30	**Maja Stojiljkovic**, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia Diagnosis of rare diseases – an NGS approach
16.30—17.00	**Jernej Kovač**, Center for Medical Genetics, University Children's Hospital, University Medical Centre Ljubljana, Slovenia Introduction of NGS technology in a routine genetic diagnostics setup: Experience from Slovenian paediatric population
17.00—17.30	**Natasa Tosic**, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia Genetic profiling of haematological malignancies
17.30—18.00	**Borut Peterlin**, **Ales Maver**, Clinical Institute of Medical Genetics, University Medical Center Ljubljana, Slovenia NGS in Slovene health system improves diagnosis of rare diseases
18.00—18.30	**Emilio J. A. Roldan-Prinsich**, Scientific Direction, Gador SA, Buenos Aires, Argentina NGS expediting Orphan Drug Developments: The need of fast global drug availability, accessibility and equity
19.30	Welcome reception

DETAILED PROGRAMME

DAY 2 WEDNESDAY, 4. MAY 8.30-19.30

8.30—9.00	Meet the speakers **Vita Dolžan, Samo Ribarič**, Faculty of Medicine, University of Ljubljana, Slovenia Presentation of Artemida project
	Pharmacogenomics and pharmacoepigenomics **Chairpersons: Kristel Van Steen, Vita Dolžan**
9.00—9.45	**Kristel Van Steen**, Université de Liège, Belgium Reductionism and complexity in the omics era
9.45—10.30	**Tanja Kunej**, Biotechnical Faculty, University of Ljubljana, Slovenia Potential of microRNAs for biomarker development
10.30—11.00	Coffee break
11.00—11.30	**Marianna Lucafò**, University of Trieste, Italy Role of non-coding RNAs in glucocorticoid response
11.30—12.00	**Nadja Kern Prezelj**, VWR International gmbH QuantaBio Innovative qRT-PCR, qPCR and miRNA technologies (sponsor lecture)
12.00—12.30	**Fabian Kuck**, Biotek CytationTM Cell Imaging Multi-Mode Reader: An Advanced Tool for Smarter Target-Based and Phenotypic Screening (Kemomed sponsor lecture)
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14.00—14.40	Poster session

	Pharmacogenetics and personalized medicine **Chairpersons: Ron van Schaik, Nada Božina**
14.40—15.20	**Ron van Schaik**, Erasmus MC, Rotterdam, The Netherlands Pharmacogenetics in Transplantation: what can we use?
15.20—16.00	**Giuliana Decorti**, University of Trieste, Italy Therapy personalization of IBD
16.00—16.30	Coffee break
16.30—17.00	**Nada Božina**, Clinical Institute of Laboratory Diagnosis, Zagreb University School of Medicine, Croatia Pharmacogenetic testing in psychiatry
17.00—17.30	**Vita Dolžan**, Faculty of Medicine, University of Ljubljana, Slovenia Pharmacogenetics of Type 2 diabetes treatment
17.30—17.50	**Lana Ganoci**, University of Zagreb School of Medicine, Croatia Clinical implementation of pharmacogenetics in Croatia
17.50—18.10	**Ernest Tambo**, Universite des Montagnes, Cameroon Developing and promoting genetics and genomic medicine integration and uptake strategies in Africa
18.10—18.30	**Nikola Kotur**, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia Maintenance therapy of childhood ALL patients induces TPMT gene expression in VNTR dependent manner
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DETAILED PROGRAMME

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8.30—9.00	Meet the speakers **Nada Božina**, Clinical Institute of Laboratory Diagnosis, Zagreb University School of Medicine, Croatia Integrating Pharmacogenomics with Pharmacovigilance – Croatian Experience **Oxidative stress and disease, Gene-environment interactions** **Chairpersons: Antonio Cuadrado, Irina Milisav**
9.00—9.45	**Antonio Cuadrado**, Autonomous University of Madrid, Spain Redox Biology in Translational Medicine
9.45—10.30	**María Monsalve**, Instituto de Investigaciones Biomédicas Alberto Sols, Spain Oxidative stress induces loss of pericyte coverage and vascular instability in PGC-1alpha deficient mice
10.30—11.00	Coffee break
11.00—11.40	**Kristel Van Steen**, Université de Liège, Belgium Living in a world of interactions
11.40—12.10	**Irina Milisav**, Faculty of Medicine, University of Ljubljana, Slovenia Oxidative protein modifications in diabetes and neurodegenerative diseases
12.10—12.30	**Alenka Franko**, Clinical Institute of Occupational Medicine, University Medical Centre, Ljubljana, Slovenia Gene environment interactions: the case of asbestosis
12.30—14.00	Lunch break
14.00—16.00	The needs and directions for research and development services in pharmacy and biomedicine: round table discussion Chairpersons: **Tamara Lah Turnšek, Metka Filipič**
16.00—16.30	Coffee break
16.30	Excursion to Piran
19.30	Dinner in Piran, restaurant Tri vdove

DAY 4 FRIDAY, 6. MAY 8.30-19.30

8.30—9.00	Meet the speakers **Damjana Rozman**, Faculty of Medicine, University of Ljubljana CASyM and Systems Approaches to Tackle Multifactorial Pathologies **Bioinformatics and biostatistics for translational research** **Chairpersons: Kristel Van Steen, Matthias Samwald**
9.00—9.45	**Magnus Ingelman-Sundberg**, Karolinska Institute, Sweden Rare genetic variants do play a major role for interindividual variation in drug response
9.45—10.30	**Noël Malod-Dognin**, Imperial College London, UK Network Data Integration Enables Precision Medicine
10.30—11.00	Coffee break **Emerging technologies in pharmacogenomics**
11.00—11.30	**Janez Mavri**, Institute of Chemistry, Slovenia Introduction to Multiscale Simulation of Enzyme Catalysis: Application to Monoamine Oxidase Catalyzed Decomposition of Biogenic Amines
11.30—12.00	**Matthias Samwald**, Medical University of Vienna, Austria One man's *1 is another man's *13? Trouble with current nomenclatures in personalized medicine
12.00—12.30	**Peter Jacobs**, Thermo Fisher Scientific Emerging technologies in pharmacogenomics (sponsor lecture)
12.30—14.00	Lunch break
14.00—14.40	Poster session **Cancer genomics** **Chairpersons: Erika Cecchin, Núria Malats**
14.40—15.20	**Erika Cecchin**, CRO Aviano, Italy Clinical implementation of host pharmacogenomics: focus on colorectal cancer
15.20—16.00	**Núria Malats**, CNIO, Madrid, Spain Molecular epidemiology of pancreatic cancer
16.00—16.20	Coffee break

DETAILED PROGRAMME

New approaches to personalized cancer treatment

16.20—16.50	**Gregor Serša**, Institute of Oncology Ljubljana, Slovenia Electroporation based treatments, electrochemotherapy and electrogene therapy for treatment of cancer
16.50—17.20	**Maja čemažar**, Institute of Oncology Ljubljana, Slovenia Immunogene therapy for canine cancer: a translational model for human oncology
17.20—17.50	**Chris Allen**, Thermo Fisher Scientific A comprehensive view of Liquid Biopsy from ThermoFisher Scientific (sponsor lecture)
17.50—18.10	**Sara Gagno**, CRO Aviano, Italy Role of pharmacogenetics in metastatic breast cancer (MBC) patients treated with exemestane as first-line hormone therapy. An Italian multicentre study
18.10—18.30	**Katja Goričar**, Faculty of Medicine, University of Ljubljana Clinical-pharmacogenetic model predicting treatment outcome in malignant mesothelioma
19.30	Gala dinner

DAY 5 SATURDAY, 7. MAY 8.30-13.00

8.30—9.00	Meet the speakers **Antonio Cuadrado**, Autonomous University of Madrid, Spain

Translation to clinical practice
Chairpersons: Magnus Ingelman-Sundberg, Vita Dolžan

9.00—9.30	**Magnus Ingelman-Sundberg**, Karolinska Institute, Sweden Pharmacogenomic and epigenomic biomarkers for prediction of drug response
9.30—10.00	**Núria Malats**, CNIO, Madrid, Spain EUPancreas - An integrated European platform for pancreas cancer research: from basic science to clinical and public health interventions
10.00—10.30	**Nada Božina**, Clinical Institute of Laboratory Diagnosis, Zagreb University School of Medicine Genotype-based dosing of oral anticoagulants in clinical practice
10.30—11.00	Coffee break
11.00—11.30	**Vita Dolžan**, Faculty of Medicine, University of Ljubljana, Slovenia Preemptive testing in pharmacogenomics
11.30—12.00	**Matthias Samwald**, Medical University of Vienna, Austria Bridging theory and practice: Clinical decision support systems for personalized medicine

Closing plenary lecture

12.00—13.00	**Professor Sir Munir Pirmohamed**, MRC Centre for Drug Safety Science and Wolfson Centre for Personalised Medicine and Institute of Translational Medicine, University of Liverpool, UK Genomics and Therapeutics: The Opportunities
13.00	Closing ceremony

TUESDAY, 3. MAY

Satellite Workshop: In vitro and in vivo models for development of novel diagnostic and therapeutic approaches

Novel in vivo like in vitro spheroid systems for prediction of drug response, long term adverse reactions and for studies of liver disease

Delilah F.G. Hendriks, Magnus Ingelman-Sundberg
Department of Physiology and Pharmacology,
Karolinska Institutet, SE-171 77 Stockholm, Sweden

The liver is a vital organ for synthesis and detoxification. Novel regenerative therapies and the development of in vitro assays of liver function, are currently hampered by the rapid de-differentiation of hepatocytes in vitro. We developed novel systems of hepatocyte spheroids for long-term cultivation and characterized them using an unbiased proteomic assessment. Proteomic analyses show that the PHH spheroid cultures closely resemble in vivo liver tissue, whereas proteomes from the same donors are dramatically remodeled in 2D monolayer cultures. The spheroid systems were suited for long term toxicity studies and e.g. a drastic fialuridine toxicity occurred only after several weeks. Using in silico analyses, significant perturbations of metabolic and signaling pathways were detected in 2D monolayer cultures, while these were maintained in spheroids. PHH spheroids maintained their morphology, cell viability, periportal/perivenous zonation and functionality for 5 weeks in culture. We also found that the spheroid system can be used as a model for liver diseases such as cholestasis, steatosis and viral hepatitis. In conclusion we think that this in vitro system can be used indeed for drug target validation, analyses of mechanisms behind liver diseases and for prediction of drug metabolism, drug action and adverse drug reactions.

HepG2CDKN1A–DsRed biosensor system for rapid and simple detection of genotoxic agents

Metka Filipic
National Institute of Biology, Ljubljana, Slovenia

The regulatory requirements for genotoxicity testing rely on a battery of genotoxicity tests, which generally consist of bacterial and mammalian cell assays for detection of gene mutations and chromosomal aberrations. However, these methods are time consuming and expensive, and they require relatively high quantities of the test material. Thus, they are not suitable for screening purposes when large numbers of samples have to be tested in a short period of time. We have developed a new cell-based biosensor system that provides rapid and simple detection of genotoxic substances. This is based on stable transfection of human hepatoma HepG2 cells with a plasmid that encodes the red fluorescent protein DsRed2 under the control of the CDKN1A promoter (HepG2CDKN1A-DsRed cells). As the major downstream target gene of activated TP53, the tumour-suppressor gene CDKN1A is responsible for cell-cycle arrest following DNA damage, and it has been shown to be specifically up-regulated by genotoxic carcinogens. The assay is optimised for a 96-well microplate format and spectrofluorimetric quantification of induced DsRed expression. The assay was evaluated by testing direct-acting and indirect-acting genotoxic compounds with different mechanisms of action, along with non-genotoxic compounds. Of 25 compounds that are known to be genotoxic in vitro and in vivo, 21 (84%) were detected as positive at non-cytotoxic doses, whereas of 12 compounds not considered genotoxic, 11 (92%) were negative. These data indicate the high sensitivity and specificity of our biosensor system. The main advantages this test system are: (i) the use of metabolically competent human cells that allow the detection of most of the indirect-acting genotoxic compounds without the use of exogenous metabolic activation; and (ii) the use of spectrofluorimetric measurements of the fluorescent DsRed reporter protein in microplates, which provides easy handling and rapid data acquisition. Based on its simplicity and sensitivity, this biosensor developed with HepG2CDKN1A-DsRed cells has the potential to become a valuable tool for genotoxicity screening for chemical safety evaluation, as well as for environmental and occupational monitoring of exposure to genotoxic agents and their complex mixtures.

Experimental Oncology in vitro to in vivo: Zebrafish Model

Tamara Lah Turnšek
Department of Genetic Toxicology and Cancer Biology,
National Institute of Biology, Ljubljana, Slovenia

Translational medicine builds on basic research advances - studies of biological processes using simple and recently multiple cell cultures, followed by animal models - and uses them to develop new therapies or medical procedures. The translation from animals to human is becoming an increasing problem, due to the awareness of the need to prevent animal suffering and high costs. Therefore, new technologies for working with zebrafish are evolving rapidly [1] and opening new opportunities to contribute to improved drug development. Zebrafish models are already making a difference in a number of biomedical areas in understanding disease progression and treatment. Examples of success in cardiology and cardiotoxicity, diseases of the neuro-system, and metabolic disorders will be presented, as some of the most promising areas for further application. Last but not least important is cancer research, where the given examples will include leukemia and glioma progression [2,3]. Recent studies have also pointed out on limitations due to identification of crucial differences between human ad zebrafish biology. However, it is hoped that the less costly and highly informative zebrafish model approach, though technically demanding, will accelerate the emergence of the so called precision medicine.

References:
1. MacRae CA, et al. (2015) Nat Rev Drug Discov; 14:721-31.
2. Vittori M, et al. (2015) Histochem Cytochem; 63:749-61.
3. Vittori M, et al. (2016) R&O; 1–24.

Skeletal muscle cells as a model for identification of novel targets for insulin resistance/type 2 diabetes treatment: to starve or not to starve?

Sergej Pirkmajer[1], Alexander V. Chibalin[2]
[1]Laboratory for Molecular Neurobiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Zaloska 4, 1000 Ljubljana, Slovenia
[2]Integrative Physiology, Department of Molecular Medicine and Surgery, Karolinska Institutet, von Eulers väg 4, SE-171 77 Stockholm, Sweden

Skeletal muscle is a major site of insulin resistance in type 2 diabetes and a key target tissue for development of novel anti-diabetic drugs. Screening for identification of novel pharmacological targets in skeletal muscle is frequently first performed in cultured skeletal muscle cells, one of the most widely used models for investigation of molecular mechanisms underlying insulin resistance and type 2 diabetes. Major advantages of using cultured skeletal muscle cells are well-controlled experimental conditions as well as easy manipulation, low cost, and reduced use of experimental animals. One of major disadvantages is the artificial cell culture environment, which may modulate physiological and pharmacological properties of skeletal muscle cells.

Under standard protocol skeletal muscle cells are cultured in various basal media supplemented with different concentrations of serum. Concentrations of serum usually range between 2% and up to 20%, depending on the type of medium and the differentiation stage of skeletal muscle cells. Serum is thus a major constituent of media used for culturing skeletal muscle cells. On the one hand, addition of serum, which contains growth factors and hormones, provides optimal conditions for growth and differentiation of skeletal muscle cells. However, on the other hand, serum also contains a plethora of proteins and other substances with unknown or poorly defined functions. Moreover, serum composition is not fixed and may vary considerably between different suppliers and even between different batches of serum from the same supplier. Despite such variability composition of serum is not determined routinely. Serum thus contains known or unknown substances in unknown concentrations. These substances may have profound effects on physiological and pharmacological properties of cultured skeletal muscle cells. Clearly, serum is a prime source of known unknowns as well as unknown unknowns, which increase experimental variability in unpredictable ways.

To circumvent such experimental and analytical uncertainties experiments in cultured skeletal muscle cells are often performed in the absence of serum. The procedure of incubating cells in the absence of serum is usually referred to as serum starvation. The usefulness of serum starvation in skeletal muscle research goes back to the classical experiments by Amira Klip and her collaborators [1], who showed that physiological insulin-stimulated glucose uptake can be observed in serum-starved skeletal muscle cells. Conversely, insulin-stimulated glucose uptake was almost completely obscured in skeletal muscle cells exposed to standard concentrations of serum. Aside from metabolic assays, serum starvation is widely used for investigation of intracellular signalling in response to endogenous or pharmacological compounds. In this context, one of the most frequently stated reasons for performing serum starvation is the reduction of basal signalling activity under serum-starved conditions. For a signalling assay low basal signalling activity in serum-starved cells would be particularly desirable because it would increase sensitivity as well as dynamic range of the assay. However, we showed that serum starvation in primary human skeletal muscle cells, L6 skeletal muscle cells and HEK293 cells induces rapid, dynamic and divergent responses across different signalling pathways [2]. We also demonstrated that serum starvation does not produce uniform reduction in basal activity of signalling pathways. Thus, a sudden removal of serum represents a major disturbance that sets off a plethora of divergent signalling responses in cultured cells and can therefore potentially interfere with the experimental results and subsequent conclusions.

In sum, while serum starvation has proved to be an indispensable procedure for various metabolic, pharmacological, and signalling studies, removal of serum can affect cellular phenotype, blunt or augment responses to various stimuli or even completely alter the result of an experiment. Serum starvation will undoubtedly remain an essential procedure for identification of novel pharmacological targets in skeletal muscle; however, interpretation of results obtained from experiments in serum-starved skeletal muscle cells should be subject to constant scrutiny and evaluation.

References:
1. Klip, A., Li, G., et al. (1984). Am J Physiol 247, E291-296.
2. Pirkmajer, S., et al. (2011). Am J Physiol Cell Physiol 301, C272-279.

Models of neurodegenerative diseases

Antonio Cuadrado
Autonomous University of Madrid, Madrid, Spain

Neurodegenerative diseases represent the most important societal challenge to balance health span with life span in countries with aged population such as most European countries. In spite of the efforts made in the last few years, the etiology of these diseases remains largely unknown and the therapeutic strategies to stop disease progression are still lacking. It is clear that superior animal models of neurodegenerative diseases are needed. Our view is that the proteinopathy that characterizes these diseases (synuleinopathy, tauopathy, amyloidopathy) represent endpoints of this disease for the vast majority of cases which are characterized by a slow progression and clinical emergence in the elderly. This may explain why the plethora of currently used animal models of have provided so far an incomplete view of these diseases and why pharmaceutical pipelines have consistently failed to translate promising preclinical observations to a clinical setting. In other words, "curing" transgenic proteinopathy in otherwise healthy mice is not enough to understand late onset of neurodegenerative diseases or to endorse an effective neuroprotective therapy. In this lecture we will present as an example, a preclinical study aimed at providing superior models of neurodegeneration that combine synuleinopathy, as hallmark of Parkinson's disease, with deficiency of the transcription factor NRF2, a master controller of many homeostatic genes. Pharmacological activation of NRF2 was achieved at the basal ganglia by repurposing dimethyl fumarate (DMF), a drug already in use for the treatment of multiple sclerosis. Daily oral gavage of DMF protected nigral dopaminergic neurons against Ð-synuclein toxicity. This neuroprotective effect was correlated with altered regulation of autophagy markers and with a shift in microglial dynamics towards a less pro-inflammatory and more wound-healing phenotype. In postmortem samples of PD patients, the cytoprotective proteins associated with NRF2 expression, NQO1 and p62, were partly sequestered in Lewy bodies, suggesting impaired neuroprotective capacity of the NRF2 signature. These experiments provide a new strategy to develop animal models for neurodegenerative diseases and new approaches to drug development.

Clinical Implementation: do YOU have your DNA passport?

Ron HN van Schaik
Dept. Clinical Chemistry, Erasmus MC Rotterdam,
The Netherlands

Interindividual variation in drug metabolism is a factor affecting successful drug therapy. Adverse drug reactions are responsible for 5-7% of hospitalizations each year, and there is thus a need to personalize drug therapy. With the knowledge and tools available today, we can achieve this at reasonable costs. With over 5,000 articles per year currently being published on genomic markers to guide drug therapy, there is a hughe potential yet the clinical implementation is still slow. Several tests have been accepted by the clinic, whereas others have not. Surprisingly, these acceptations may differ from country to country, but also from clinic to clinic.

Our own experience in implementing pharmacogenetics in clinical diagnostics will be illustrated, based on our 10 year experience. This implementation strategy covers education, availability of testing, laboratory and clinical guidelines, quality, feedback from clinicians and patients, reporting, financial and ethical aspects, networking and interlaboratory collaborations. Current status in the Netherlands, as well as encountered and unexpected barriers will be addressed.

At Erasmus MC, we provide since 2015 DNA passports, fitting in the trend of pre-emptive genotyping. With this passport, one can visit any pharmacy in the Netherlands to obtain medication adjusted on genomic profile for over 80 drugs. The question is, therefore: "Do YOU have your DNA passport ready?"

Diagnosis of rare diseases – an NGS approach

Maja Stojiljkovic, Sonja Pavlovic
Institute of Molecular Genetics and Genetic Engineering,
University of Belgrade, Belgrade, Serbia

Rare diseases are a very heterogeneous group of medical conditions. Each rare disease affects small number of people compared to the general population (1 person per 2000), but patients are numerous given that more than 6000 rare diseases have been described. Specific issues are raised in relation to their rarity, such as lack of diagnostic procedures and lack of treatment, giving the rational for putting together such a numerous and diverse group.

Majority of rare diseases, 80% of them are genetic diseases and therefore identification of specific gene defect in each patient is important. Early diagnosis is important to improve quality of life and provide appropriate treatment when possible. Identifying the genetic background of patients with rare diseases may help in their counseling and that of their relatives. It is crucial to select a suitable genetic test, which includes all genes associated with symptoms and all types of possible genetic changes. For many years, Sanger sequencing have been used in clinical testing and was considered gold standard method for genetic diagnosis. Thus, we were performing Sanger sequencing of RNA-coding exons and flanking splice sites regions for several monogenic diseases, like beta-thalassemia and phenylketonuria. However, limitations of this approach are low throughput and high cost, and analyzing large genes and multi-gene panels is laborious and expensive. Using next generation sequencing (NGS) for simultaneous analysis of all genes responsible for development of symptoms perceived in a patient, all clinically relevant genes or the whole genome has increasingly become method of choice for molecular genetic diagnosis of rare diseases. In addition, ability of NGS to detect mutations in large genes and to identify copy number variations is very advantageous.

In order to diagnose different rare diseases in Serbia, we combined targeted gene analyses (Sanger sequencing) with simultaneous analysis of 4813 genes (clinical exome). More than 110 patients with rare diseases have been diagnosed, including patients with hyperphenylalaninemias, glycogen storage diseases, organic acidurias and mitochondriopaties. Moreover, NGS approach has proven to be irreplaceable for accurate diagnosis of diseases with overlapping clinical manifestations. For example, pathogenic variants in the PEX6 gene revealed diagnosis of a defective peroxisomal biogenesis disorder instead of lysosomal storage disease, while pathogenic variants in the SBDS gene pointed to Shwachman–Diamond syndrome diagnosis after initial suspicion of glycogen storage disease Ib. In our experience NGS approach leads to timely and accurate genetic testing, sets definite diagnosis and enables rapid implementation of optimal therapy for patients with rare diseases.

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Introduction of NGS technology in a routine genetic diagnostics setup: Experience from Slovenian pediatric population

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Introduction: Next Generation Sequencing (NGS) technology presents a typical disrupting innovation, drastically changing the fields of genomics and clinical genetics. The NGS came a long way from experimental and research tools to crucial application in genetic diagnostics laboratories inciting the development of different data analysis tools, diagnostic approaches and quality control checkpoints to support and promote clinical application of NGS technology. Established NGS diagnostic protocols and algorithms of orphan and common diseases identification and diagnostics will be presented, together with the quality assurance checkpoints introduced into the routine work of USLD.

Results: Since 2014, USLD adopted and developed several approaches to utilize NGS technology in the routine genetic diagnostics of orphan and common disease. More than 600 pediatric patients and their parents or siblings underwent the NGS diagnostics in the past 2 years with referral reasons spanning from relatively common traits (hypercholesterolemia and obesity) to specific, rare traits (epileptic encephalopathy, hearing loss). Algorithms supporting analysis of such a diverse phenotypes encompass utilization of different analytical approaches from family trio analysis to phenotype ontology analysis. Additionally, the NGS analysis was successfully coupled with universal population screening for hypercholesterolemia, improving the detection rate and significantly reducing time to final diagnosis as well as demonstrating the power of biochemical phenotype supported NGS analysis [1]. Moreover, patients with orphan diseases benefited as well with increased diagnostic rate and reduced turnaround time even for the most unexpected genetic causes of their disease [2].

Conclusions: The NGS drastically changed the work of USLD genetic laboratory, introducing new paradigms of genetic data analysis as well as expanding the spectrum of clinical phenotypes referred to genetic diagnostics. The NGS technology proved to be an invaluable tool in the diagnostics of complex traits where hundreds of genes may influence the clinical phenotype. Nevertheless, stringent quality control parameters from gDNA quality analysis, depth of sequencing analysis to utilizing Sanger sequencing to confirm NGS findings, are necessary to assure the highest possible quality and accuracy of the results.

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Genetic profiling of haematological malignancies

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Introduction: Hematological malignancies are a heterogeneous group of the disease, which is a reflection of their great genetic diversity. They are of clonal origin, and the transformation from normal to malignant cell occurs as a result of gradual accumulation of mutations in a number of genes involved in basic cellular processes. One of the main goals in the study of hematological malignancies is the definition of relevant genetic markers, mutations that are crucial for the development of the disease. Development of next generation sequencing (NGS) technology has provided a possibility to obtain a large amount of mutation data. In that way the researchers gained more comprehensive insight into the origin and evolution of carcinogenesis in the pathogenesis of hematological cancers.
In our study we applied amplicon based NGS approach, using TruSeq Amplicon Cancer Panel (TSCAP) for 48 cancer-related genes (Illumina, Inc). We analyzed DNA samples from 19 patients with primary diffuse large B-cell lymphoma of central nervous system (DLBCL CNS), as well as, DNA samples from 20 childhood acute myeloid leukemia (cAML) and 20 adult acute myeloid leukemia (aAML) patients.

Results: In 19 primary DLBCL CNS patients, we identified a total of 920 variants in the coding regions (median per patient: 43, range: 6-122). When potentially protein-changing mutations (nonsense (N), frameshift (F) and missense (M) alterations) were analysed, a total of 559 mutations within coding regions (median per patient 28) were found. Four patients had over 50 NFM mutations and in at least 5 from 19 cases, we identified 28 genes containing potentially protein-changing mutations. Over 80% of NFM mutations, were detected in 8 genes: CTNNB1, PIK3CA, PTEN, ATM, KRAS, PTPN11, TP53, and JAK3, pointing to a potential role of these genes in lymphomagenesis.
In the analysis of cAML and aAML patients, we identified a total of 412 variants in the coding regions (median per patient 10 in both groups). A total of 122 variants, 62 cAML and 60 aAML were potentially protein-changing. Six patients had 5 or more NFM mutations, including 3 cAML and 3 aAML. Our analysis identified 4 cAML-specific genes (JAK3, ABL1, GNAQ and EGFR) and 5 genes containing NFM mutations only in aAML patients (IDH1, APC, HNF1A, GNAS and SMARCB1).

Conclusions: Our results showed that AML contains relatively small number of genetic alterations, suggesting that for the development of AML fewer genetic alterations are required than for other hematological malignancies like lymphoma. Application of modern NGS technology in our study resulted in the information about genetic profile of each patient individually. By identifying genetic changes specific to each patient and by application of targeted therapies directed towards the annulment of the given mutation effects, leads to highly specific personalized therapy of hematological malignancies.

NGS in Slovene health system improves diagnosis of rare diseases

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Diagnostics in the field of rare genetic diseases presents a significant challenge in assuring comprehensive, equally accessible and high quality diagnostic service on a national level.

We present an innovative model towards diagnostics of heterogeneous genetic disorders, which is based upon emerging genomics technologies, with specific stress on the utilisation of next-generation sequencing (NGS) in clinical practice. With the establishment of Centre for Mendelian genomics in 2013, we have made available a harmonised pathway to genetic diagnostics through mendeliome, exome and genome sequencing. Using high-throughput sequence analysis, tailored pipelines and extended analysis of sequencing data in over 1000 cases with suspected genetic etiology, we were able to reach total diagnostic yield of 39.5%, which we were able to increase this yield to 43.5% using methods of extended exome analysis.

We have successfully transitioned patient information collection, analysis and reporting to standardised phenotype nomenclature (in accordance to Human Phenotype Ontology nomenclature). We provide presentation and assessment of phenotype driven-approach to interpretation of genome-level data and present our approach to phenotype-driven diagnostic process in rare diseases.

Our experience to date shows that the transition to new generation of genetic tests has improved the diagnostic yield, reduced the overall per-case resources for reaching final diagnosis and also reduced the need for cross-border genetic testing.

NGS expediting Orphan Drug Developments: The need of fast global drug availability, accessibility and equity

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The early years of the current century in biomedicine were driven by genomics and epigenetic developments. Their applications in human health are levered by NGS which in turn is changing medical paradigms. Nevertheless, it is essential that these changes be organized so as they turn knowledge into available, accessible, and with an equivalent quality of health assistance worldwide. In this task, every single NGS operator/researcher plays a key role as it has been repeatedly demonstrated in the rare diseases (RDs) field. RDs are a group of around 7,000 conditions having in common the fact that they are not well known by practitioners, poorly investigated and with low priority at health policy makers in many countries. Conversely, researching RDs will provide substantial information to fill the epigenomic maps in connection with common diseases (connectome) and will also facilitate the understanding of the pathogenic pathways toward repurposing of drugs, one of the formulas for making health cost more accessible. Hence, the application of NGS to RDs diagnosis and research is envisioned as one of the most promising fields of the current biomedical field. In spite of counting with few resources (proportionally), the structuration of working networks, open access data-bases, and collaborative organizations is tracking and expediting the RDs and orphan drugs developments. Hence, NGS activities should be properly introduced into master plans, international collaboration and the join teamwork with members of the government's, industries and patient groups. The absence of any of these three variables will make the individual contributions and efforts frustrating, as it had been sadly experienced by the pioneers in the field. In addition, the NGS operator/researcher will soon enter a new scenario of communication tools, information management, intellectual property and health policy criteria adjusted specifically to the rare diseases needs. Therefore, together with the necessary specialization in NGS technical aspects, a responsible and convenient formation in RDs as well as in orphan drugs and diagnosis, are essential to become part of the most advancing and innovative projects in the coming years. Then, accessibility to orphan products, which today is challenged by high costs, limited productions, lack of knowledge and opportunities in RDs diagnosis becomes as a major task for the collaborative groups. Also, without any accessibility plan, the ethics of the projects or plans are debatable. In conclusion the present and near future are widely open for NGS, but the immediate needs from patient groups, health policy makers and industry, and the operating changes at the current scenario should be understood in order to be part of the benefits. The individual responsibility and will to collaborate are firm characters which should be early incorporate in the daily task of professionals working with NGS.

WEDNESDAY, 4. MAY

Pharmacogenomics and pharmacoepigenomics

Reductionism and complexity in the omics era

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Whereas analytic reductionism is often seen as the opposite of analytic holism, molecular biology and systems biology are becoming increasingly intertwined. Here, we show that in "systems thinking" there is a place for both viewing systems and their properties as a whole and not as collections of parts, and for viewing complex systems or phenomena by their simpler components. We illustrate this via two examples. The first example is taken from the field of "data integration". Data integration, whether restricted to omics, or to the environment, or both requires following rigorous standard operating procedures. Once environmental omics data have been curated, the literature often reports about the following three main routes of analysis: either the data are fused prior to modeling, or the representation of each data sources is altered to make it more digestible prior to deriving an integrative solution to the problem of interest, or each data set is modeled separately and results are integrated. We will show the extra benefits that can be gained by following alternative routes, driven by out-of-the-box thinking. The second example is taken from "translational systemics". Here, we aim to create awareness that, in order to bring about personalized medicine in clinical practice, there is a need to look at a larger "whole" to act as context.

Potential of microRNAs for biomarker development

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MicroRNAs (miRNAs) are a class of short non-coding RNAs involved in the regulation of gene expression. It has been estimated that these molecules fine-tune the expression of around 30% of protein-coding genes either by their up- or downregulation. On average each miRNA is predicted to regulate 200 targets. They are part of the complex regulatory network and are associated with several epigenetics concepts. For example, miRNA silencing is one of the classes of epigenetics mechanisms, additionally, miRNA genes themselves could also be epigenetically regulated and a group of miRNAs (epi-miRNAs) can directly target genes encoding for epigenetic machinery such as DNA methyltransferases and histone deacetylases [1]. MicroRNAs have been shown to be involved in numerous physiological processes as well as disease development. They have a potential for diagnostic and prognostic biomarkers as well as treatment targets. They have also been shown down-regulate genes that are important for drug function. However, finding a miRNA candidate for functional studies still presents a challenge because understanding of complex miRNA related interactions is not yet complete. Additionally, the field lacks central miRNA genomics repository and the data are fragmented through various databases and publications. Several bioinformatics tools are missing and many of the existing tools are not regularly updated due to constant updates of the source databases. Various possible directions for miRNA based biomarker prioritization exist. One of the strategies is to first develop an integrated atlas of miRNA gene regulatory elements, consisting of known upstream regulators, downstream targets, overlapping genomics elements, genetic variations and phenotypic associations. Biomarker selection could then be performed based on integration of various omics levels, including DNA level, transcriptomics, interactomics and pharmacogenomics. One of the possible strategies for biomarker development is integrated analysis of heterogeneous gene expression profiles for development of robust disease-specific transcriptional fingerprints. Next, potential biomarkers could be located within miRNA regulatory regions (miR-rSNPs), mature miRNA regions (miR-SNPs), miRNA target sites (miR-TS-SNPs) or within genes encoding for miRNA silencing machinery (miR-SM-SNPs), such as DROSHA and DICER1 [2]. For pharmacogenomics studies it is necessary to identify triplet miRNA-target-drug sets. Understanding of complex interplay between miRNAs, other classes of non-coding RNAs and protein-coding genes is not yet complete, but is of importance for development of novel biomarkers and for the design of more effective therapeutic strategies.

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Role of non-coding RNAs in glucocorticoid response

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Natural and synthetic glucocorticoids (GCs) are widely employed in a number of inflammatory, autoimmune and neoplastic diseases, and, despite the introduction of novel therapies, remain the first-line treatment for inducing remission in inflammatory bowel diseases (IBD). These agents exert their biological effects through the binding to the GC receptor (GR), which translocates from the cytoplasm into the nucleus and binds, through its DNA-binding domain (DBD), the glucocorticoid responsive elements (GREs) in the regulatory regions of GC responsive genes.

Considerable clinical evidence of wide inter-individual differences in GC efficacy and side effects among patients has been reported. The mechanisms involved in GC resistance are scarcely understood and there is presently no means to predict the response in advance.

In this context, non-coding RNAs, including both long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), represent a new and promising field of research. LncRNAs are non-protein coding transcripts longer than 200 nucleotides and they act as regulators at different levels of gene expression, including chromatin organization, transcriptional regulation, and post-transcriptional control. miRNAs are small (18-24 nucleotides) non-coding RNAs that function as guide molecules in RNA silencing.

Recent results obtained in our laboratory suggest a role for the lncRNA growth arrest-specific 5 (GAS5) in modulating GC response in peripheral blood mononuclear cells (PBMCs). GAS5 interacts with the activated GR, preventing its association with GREs, and consequently suppressing its transcriptional activity [1]. We have demonstrated that PBMCs resistant to GCs express higher levels of GAS5 in comparison with good responders, and hypothesized that upregulation of GAS5 could interfere with GR activity, leading to the resistance phenotype observed [2-3]. If these results are confirmed in a larger series and in patients with chronic inflammatory and autoimmune diseases, GAS5 should be considered as a candidate marker of GC resistance.

There is a lot of interest in identifying the role of miRNAs in the modulation of genes involved in drug response, and to date, no data are reported about miRNA regulation by GCs in IBD [4]. In order to identify miRNAs deregulated by GC treatment we have obtained high-throughput miRNA profiles in paediatric IBD patients at diagnosis and after 4 weeks of steroid treatment and we have detected miRNAs containing GREs for a potential direct regulation by the GR. These results could represent a first step for their translation as pharmacogenomic biomarkers.

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QuantaBio Innovative qRT-PCR, qPCR and miRNA technologies

Nadja Kern Prezelj, Territory manager for
Slovenia and Life science Specialist
VWR International gmbH

Quanta Biosciences is now Quantabio! Quantabio develops and manufactures the most advanced DNA/RNA amplification reagents available today. Our technologies are widely utilized in applied industry, clinical laboratories, and life sciences research.

Our reagent portfolio continues to set new standards in critical PCR- based assay performance and reagent supplier reliability. Our innovative technologies for which we are well known include:

- qScript® reverse transcriptase for reliably generating cDNA suitable for qPCR,
- ToughMix® additives for overcoming common PCR inhibitors,
- qScript microRNA cDNA Synthesis Kit for highly-sensitive reverse transcription of small, non-coding RNA, followed by precise quantification of microRNAs.

Evaluation of quantitative miRNA expression platforms in the microRNA quality control (miRQC) study, published in Nature Methods [1], confirmed Quantabio's miRNA technologies are outperforming in accuracy and reproducibility.

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Cytation™5 Cell Imaging Multi-Mode Reader: An Advanced Tool for Smarter Target-Based and Phenotypic Screening

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Cell-based assays are de rigueur in understanding disease biology and its intervention through drug discovery efforts. These assays have been facilitated by the establishment of myriad cell lines often derived from human patients with various cancers. These cell lines are relatively easy to maintain in culture indefinitely while maintaining their particular phenotypes. Over the last decade, there has been a movement to refine cell models of disease to higher biological relevance, which includes a reliance on endogenous expression of drug targets and their substrates, the use of primary cells instead of cell lines, and a return to phenotypic assays that do not rely on the modulation of a single drug target for disease intervention.

This wide array of new cell-based assays and methods requires diverse methods of detection. As an example, using endogenous expression of proteins associated with disease requires high sensitivity detection; while phenotypic assays are commonly performed with devices that image the cells. In this treatment, we will describe the utility of PMT- and CCD-based detection to facilitate a wide variety of cell-based assays incorporating some of the latest trends in cell-based assays. In particular, we will show how these two modalities can function together to reduce screening time and data storage requirements that are weaknesses of multi-parametric high content screening applications.

Figure 1: Figure of Cytation 3

Pharmacogenetics in Transplantation: what can we use?

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In the field of solid organ transplantation, the success of the transplantation heavily depends on adequate immunosuppression. Whereas initially azathioprine was used as therapy, much improvement has been made by using tacrolimus/mycophenolic acid (MMF) or cyclosporine/MMF combinations. Azathioprine is metabolized by the enzyme thiopurine methyltransferase (TPMT), one of the oldest known pharmacogenetic targets. Approximately 10% of the population has an intermediate enzyme activity and 0.3% of the population even a very low activity, caused by genetic polymorphisms. A low TPMT activity will cause unexpected high levels of the active metabolites, yielding haematological toxicity which may be fatal. For tacrolimus and cyclosporine, overexposure as well as underexposure will cause either nephrotoxicity, or organ rejection, respectively. Yet, the pharmacokinetics of these drugs varies considerably between individuals, which is why therapeutic drug monitoring is being used. Both tacrolimus and cyclosporine are substrates for CYP3A4, whereas tacrolimus is also metabolized extensively by CYP3A5. As for MMF, this compound is converted to the active form mycophenolate (MPA). Currently, there is a debate whether or not there should be therapeutic drug monitoring to check MMF therapy. MPA is converted by UGT1A9 into MPA-glucuronide. Genetic polymorphisms in UGT1A9 may affect MPA pharmacokinetics as well as clinical outcome (acute rejection). The background and clinical usefulness of these pharmacogenetics targets will be discussed.

Therapy personalization of inflammatory bowel disease

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Inflammatory bowel disease (IBD) comprises ulcerative colitis and Crohn's disease; these diseases have a high diffusion and morbidity in the population worldwide, even in pediatric patients. Several therapeutic options exist for IBD, but they are ineffective in a substantial portion of patients or have an increased probability of adverse events, therefore, the perspective of pharmacological tools that allow identification of these patients is particularly desirable.

Personalized therapy approaches already available or in development for pediatric patients with IBD will be considered. Particular attention will be given to thiopurine therapy personalization. Indeed, these antimetabolites have a well-described risk benefit profile that can safely maintain at least 20% of patients in a state of stable long-term steroid-free clinical remission. However, between 20 and 30% of patients do not achieve a satisfactory clinical response during azathioprine therapy and 10-28% of patients experience side effects requiring discontinuation of therapy.

Personalization of therapy for pediatric patients with IBD, comprising pharmacokinetic, pharmacodynamic and pharmacogenetic assays will be discussed; this combined approach should help clinicians in identifying patients who will not respond to therapies, in finding patients at risk of side effects and in choosing the ideal drug, the ideal dose, and employing less expensive agents.

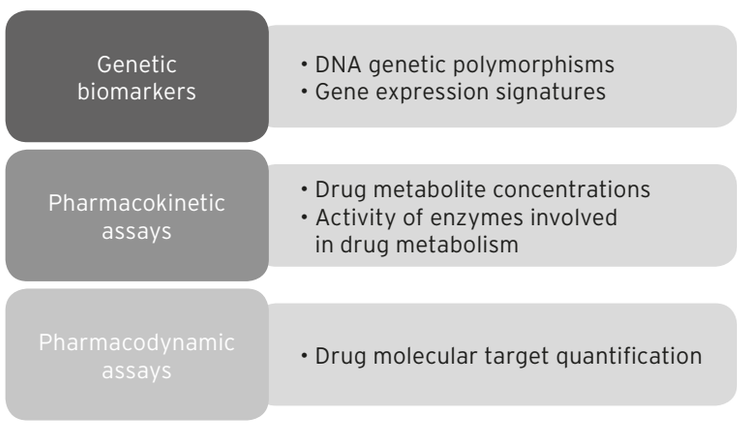

Figure 1: Pharmacological tools for IBD therapy personalization

Pharmacogenetic testing in psychiatry

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Pharmacogenomics investigations in psychiatry attempt to define the impact that genetic polymorphisms have on positive and adverse reactions to psychotropic drugs. Candidate pharmacokinetics' genes are these of drug—metabolizing enzymes and membrane drug transporters, while on pharmacodynamics' part these are genes encoding drug targets: elements of neurotransmitter pathways such as transporters and receptors. The most important enzymes involved in the metabolism of psychotropic drugs are CYP2D6, CYP2C19, CYP1A2 and CYP3A4. P-glycoprotein, coded by polymorphic MDR1/ABCB1 is recognized to be important in regulating access of therapeutic agents to the brain and other tissues, and thus has pharmacogenetic relevance. At pharmacodynamic level, polymorphic serotonin transporter (SERT), as the main target of many antidepressant drugs, could also be an attractive candidate for psychopharmacotherapy outcome assessments. Lot of research have been undertaken to provide a better understanding of the genetic factors involved in weight gain and metabolic disturbances associated with second generation antipsychotics treatment. Notwithstanding the large effects of pharmacogenetic polymorphisms on the kinetics of psychotropic drugs, individual response is still poorly characterized due to insufficient knowledge of pharmacodynamics mechanisms. Genetics is only one of many variables (environmental, personal) adding to the complexity of drug response. For some drugs, genetics may be extremely important in most patients, while in others it may be irrelevant when compared with environmental factors. Genetic testing may be crucial for understanding some rare ("outliers") patients' drug response. In psychiatry, two types of pharmacogenetic tests are ready for clinical practice: CYP2D6 and CYP2C19 testing for some antidepressants and antipsychotics, mostly for dosing, and HLA testing to rule out carbamazepine for some Asian patients. Other tests offered to psychiatrists in the US and in few European countries for genotyping of multiple CYPs and pharmacodynamic genes have limited data on clinical validity/utility. As therapeutic drug monitoring (TDM) identifies phenoconversion due to inhibitors/inducers, it is crucial to conjointly implement TDM and pharmacogenetic testing in order to advance personalized dosing. Educating health professionals in how to use pharmacogenetic tests for drug selection/dosing in the context of other factors such as environmental and personal factors and pharmacological mechanisms is important task, due to limited pharmacological training in medical schools.

Pharmacogenetics of Type 2 Diabetes Treatment

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Type 2 diabetes (T2D) is a lifetime, chronic disease associated with insulin resistance and insufficient pancreatic β-cell function in the post-prandial state. Several classes of oral antidiabetic drugs with different mechanisms of improving glycemic control, metabolic effect and adverse event profiles are available. Diet, lifestyle changes and drugs used to treat dyslipidemia and hypertension, also play an important role in preventing T2D complications. Great interindividual variability in clinical response to oral antidiabetic drugs is observed in clinical practice. This may be in part due genetic polymorphisms in genes coding for metabolising enzymes, transporters and targets of these drugs.

Sulphonylureas (SU) are insulin secretagogues. SU are metabolized by polymorphic cytochrome P450 2C9 and CYP2C9 polymorphisms were reported to influence response to SU. Polymorphisms in ABCC8 and KCNJ11 genes that encode SU target, an ATP-sensitive K(+) channel, were also associated with SU response in some studies. These polymorphisms did not influence glycemic control in a group of 156 Slovenian SU treated T2D patients, although CYP2C9 polymorphism significantly increased the risk of hypoglycaemia and the number of hypoglycemia events in elderly patients [1].

Metformin acts as insulin sensitiser that suppresses excessive hepatic glucose production and increases glucose utilization in peripheral tissues. Metformin does not undergo hepatic metabolism so its pharmacokinetics largely depends on the activity of organic cation transporter 1 (OCT1, encoded by SLC22A1) and multidrug and toxin extrusion protein 1 (MATE 1, encoded by SLC47A2). Response to metformin was associated with SLC22A1 and SLC47A1 polymorphisms in some studies. However, in a group of 135 Slovenian T2D patients on long term metformin and SU treatment, SLC22A1 and SLC47A1 polymorphisms influenced lipid status, but they had no impact on glycemic control [2].

A newer class of glucose dependent insulin secretagogues are glucagon like peptide 1 receptor (GLP1R) agonists. They also promote weight loss in obese patients by lowering food intake through their actions on intestine and central nervous system. We were the first to show association between GLP1R gene (GLP1R) polymorphisms and weight loss after treatment with liraglutide in women with polycystic ovary syndrome [3]. Elucidation of genetic variants associated with the T2D risk may also lead to identification of novel therapeutic targets.

Although it appears that pharmacogenomics has the potential to improve the management of T2DM and help clinicians in the effective prescribing of oral antidiabetic drugs, data are still scarce and not consistent enough to make any treatment recommendations.

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Clinical implementation of pharmacogenetics in Croatia

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Introduction: Pharmacogenomic testing is nowadays an integral part of precision medicine. Pharmacogenetic biomarkers present an opportunity for health professionals to improve the efficacy and safety of pharmacotherapy. Implementation of pharmacogenetic testing into the clinic is ongoing in Croatia for 17 years.

Results: Laboratory for pharmacogenetics was set up within Department of Laboratory Diagnostics at the University Hospital Centre Zagreb. Our team consist of 1FTE clinician (MD, PhD, Pharmacology Prof), 1FTE clinical chemist (PhD candidate), 1FTE technician (BCs) and 1FTE technician. We validated 30 tests (Figure 1), some with two genotyping methods (TaqMan, LightCycler, PCR, PCR-RFLP). Pharmacogenetic tests like CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, DPYD, VKORC1, TPMT are accredited according to the ISO 15189 standard. In 2015 we performed cca. 4000 analyses half of which were for outpatients. We performed collaborative research projects with clinicians in the field of psychiatry, gastroenterology, neurology, nephrology, cardiology and oncology. We organized workshops and prepared education materials for clinicians, pharmacists, clinical chemists and regulatory bodies.

Conclusions: The complexity of pharmacogenetic knowledge, from laboratory testing to the interpretation and finally prescribing the right drug is still limiting clinical implementation. Interdisciplinary approach, novel biomarkers and technologies are an imperative for further implementation.

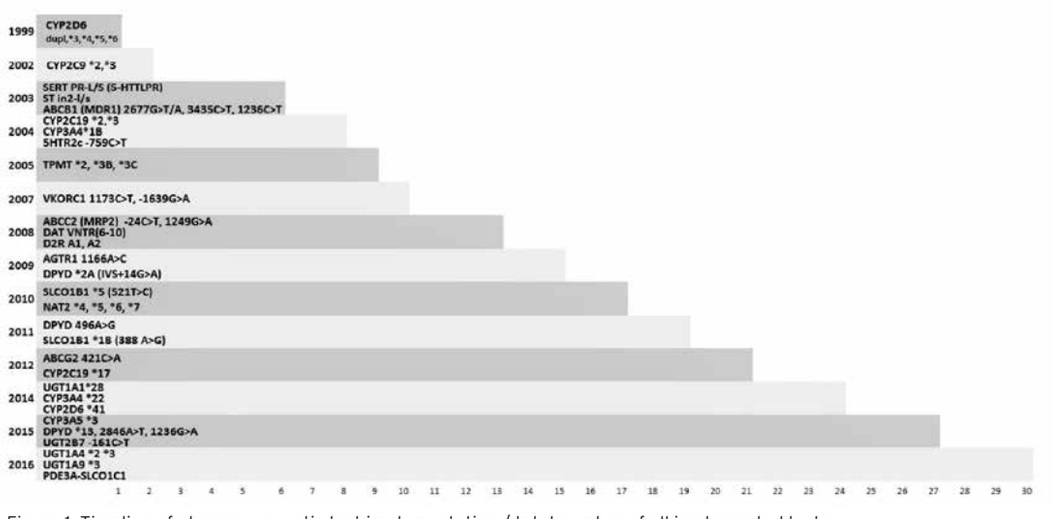

Figure 1: Timeline of pharmacogenetic test implementation / total number of all implemented tests

Developing and promoting genetics and genomic medicine integration and uptake strategies in Africa

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Globalization of trade and travel, demographic expansion, intense urbanization and epidemiological transition has accentuated the growing trend of non-communicable diseases (NCDs) burden. These continue to impose a strong drawbacks and challenges on the hard-gains from persistent infectious diseases and emerging epidemics burden on the vulnerable populations mainly those living in remote rural communities, but also on urban settings across Africa.

A systematic review was conducted in previous endeavors and research projects in clinical genetics, genomis and epigenetics, bost microbiome interaction and bioinformatics across the continent. Our findings showed that African countries and researchers are still struggling to catch up with genomic medicine scientific and technological advancements needs to tackle the cross-chair public health burden and economic impacts. Genetic testing and genetic counseling, knowledge and skills on genomic information literacy and awareness are necessary in better understanding the role and value health workers, nurses, pharmacy, physicians and other allied professionals in collective and participatory in the genomic and clinical medicine uptake and effectiveness of targeted interventions. In addition prognosis on risk factors and treatment management, and clinical practices in risk factors mitigation, lifestyle adaptations. Rapid, sensitive and point of care-based diagnostics services and counseling in genomics medicine is needed in responding to the growing trend and patterns of obesity related cardiometabolic diseases in Africa. Also in builiding common infrastructure and research capacity in mapping phenotypic associations with diabetes, hyperension, cardiovascular diseases, strolke, kidney and blood and other genetics disorders in establishing safe and efective and low cost vaccines and drug response biomarkers diverse Africa populations dissorders prevention and management.

We concluded that promoting genomic medicine in establishing clinical validity and utility of tests, establishing political commitment and investment mechanisms on how to increase awareness and promote their uptake and literacy in lifestyle adapatations, fitness and improving integration into primary healthcare service delivery in rural and urban settings in Africa.

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Maintenance therapy of childhood ALL patients induces TPMT gene expression in VNTR dependent manner

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Introduction: Thiopurine S-methyltransferase (TPMT) is the most important enzyme involved in metabolism of thiopurine drugs, such as 6-mercaptopurine (6-MP). Great variability of TPMT activity cannot be explained considering just coding region variations. Variation of regulatory elements, such as VNTR architecture (i.e. number and types of repeats) in promoter, as well as chemotherapy drugs are shown to influence TPMT enzyme activity. However, it is not known whether these factors influence TPMT gene expression in ALL patients. TPMT gene expression was measured in hematopoietic tissue of 57 childhood ALL patients, both before chemotherapy and during the maintenance therapy when 6-MP and methotrexate are administrate.

Results: Our results show that maintenance therapy strongly induces TPMT expression, more than 3 times, on average. For each ALL patient, TPMT expression was higher during maintenance therapy, than before chemotherapy ($p<10\text{-}10$). An interaction of maintenance therapy with VNTR region in TPMT promoter modified TPMT gene expression. Specifically, VNTR*5a/*5a carriers were found to be the highest expressers during the therapy ($p=0.045$), even though they were low expressers before chemotherapy. Our results confirm negative correlation between "A" repeats number of VNTR and TPMT gene expression ($rs=-0.35$).

Conclusions: Maintenance therapy strongly induces TPMT expression. This effect is modified by architecture of VNTR region of TPMT gene. It could be of great importance to consider TPMT genetic variations at the very beginning of the maintenance therapy for childhood ALL patients, especially for carriers of less expressed VNTR alleles.

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Integrating Pharmacogenomics with Pharmacovigilance - Croatian Experience

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Introduction: Integrating pharmacogenomics with pharmacovigilance (PhV) have substantial role in searching post-marketing adverse drug reactions (ADR) and in drug development. Information on genetic polymorphisms is valuable when analyzing the causal relationship between drug intake and dose related ADR.

Aim: To study the possible genetic associations with ADRs, the Croatian Agency for Medicinal Products & Medical Devices (HALMED) has piloted a project to collect DNA and phenotype data of ADR cases using the international standardized phenotypic criteria.

Methods: Besides the data from spontaneous adverse reaction reporting system, the clinical data routinely recorded in hospital settings provide additional opportunities for identifying and quantifying ADRs. Since 2010 we asked reporters of some ADRs to invite patients to participate in the study. We established biological sample repository in the University Hospital Centre Zagreb, Lab for pharmacogenomics (accredited according to ISO 15189); undertake genotyping to identify novel associations or validate findings in cohorts of patients with well-defined phenotypes.

Results: HALMED developed a method for informing physicians or pharmacists and their patients about a possible pharmacogenetic involvement in the pathogenesis of the reported ADR and for offering easy access to genotyping. Data on ADRs of statins, anticoagulants, antiepileptics, antipsychotics, immunosuppressants, NSAIDs, tyrosine kinase inhibitors, have been collected and DNAs were genotyped. An anonymized copy of the test results has been used for the interpretation of possible signals. Some study results have been published.

Conclusion: PhV centres a valuable starting point for pharmacogenomic studies and may suggest investigations and subsequent individualized pharmacogenetic counseling after a reported ADR.

Oxidative stress and disease, Gene-environment interactions

Redox Biology in Translational Medicine

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Enhanced levels of reactive oxygen species (ROS) have been associated with different disease states and therefore, consumption of ROS scavenging molecules or antioxidants as food additives and nutraceuticals has been greatly encouraged. Antioxidants may be beneficial in situations of subclinical deficiency and increased demand or acutely upon high-dose infusion. However, to date, there is little clinical evidence for the long-term benefit of most antioxidants. Alarmingly, recent evidence points even to health risks, in particular for supplements of lipophilic antioxidants. The biological impact of ROS depends not only on their quantities but also on their chemical nature, (sub)cellular and tissue location, and the rates of their formation and degradation. Moreover, ROS serve important physiological functions; thus, inappropriate removal of ROS may cause paradoxical reductive stress and thereby induce or promote disease. Any recommendation on antioxidants must be based on solid clinical evidence and patient-relevant outcomes rather than surrogate parameters. Such evidence-based use may include site-directed application, time-limited high dosing, (functional) pharmacological repair of oxidized biomolecules, and triggers of endogenous antioxidant response systems. Ideally, these approaches need guidance by patient stratification through predictive biomarkers and possibly imaging modalities. Rather than antioxidants, a new generation of protein targets for classical pharmacological agents includes ROS-forming or toxifying enzymes or proteins that are oxidatively damaged and can be functionally repaired. Linking these target proteins in future to specific disease states and providing in each case proof of principle will be essential for translating the oxidative stress concept into the clinic.

Oxidative stress induces loss of pericyte coverage and vascular instability in PGC-1alpha deficient mice

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PGC-1alpha is a regulator of mitochondrial oxidative metabolism and reactive oxygen species (ROS) homeostasis that is known to be inactivated in diabetic subjects. This study aimed to investigate the contribution of PGC-1alpha inactivation to the development of oxygen-induced retinopathy. We analyzed retinal vascular development in PGC-1alpha-/- mice. Retinal vasculature of PGC-1alpha-/- mice showed reduced pericyte coverage, a de-structured vascular plexus, and low perfusion. Exposure of PGC-1alpha-/- mice to hyperoxia during retinal vascular development exacerbated these vascular abnormalities, with extensive retinal hemorrhaging and highly unstructured areas as compared with wild-type mice. Structural analysis demonstrated a reduction of membrane bound VE-cadherin, which was suggestive of defective inter-cellular junctions. Interestingly, PGC-1alpha-/- retinas showed a constitutive activation of the VEGF-A signalling pathway. This phenotype could be partially reversed by antioxidant administration, indicating that elevated production of ROS in the absence of PGC-1alpha could be a relevant factor in the alteration of the VEGF-A signaling pathway. Collectively, our findings suggest that PGC-1alpha control of ROS homeostasis plays an important role in the regulation of de novo angiogenesis, and is required for vascular stability.

Living in a world of interactions

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"Interactions" may mean different things in different contexts. It may refer to biological interactions, genetic interactions, statistical interactions, gene-environment interactions, among others. Here, using genome-wide association interaction (GWAI) studies as starting point, we show which are the key building stones of such studies and indicate the routes to travel by for successful completion of a GWAI analysis. We create awareness of open problems and will provide pointers towards possible solutions. Then we show how lessons learned from GWAI studies can or cannot be extrapolated to the gene-environment interaction scene. Large-scale gene-environment interaction studies and large-scale gene-gene interaction (GWEI) studies, via the common genetic component they involve, share quite a number of challenges: high-dimensionality, computational capability, the absence/presence of marginal effects, the multiple testing problem, and genetic heterogeneity. We touch upon the relative roles of "genetics" and "environment", as this is important to develop the most powerful integrative nature-nurture models without losing power compared to their individual considerations. Genetics loads the gun, but the environment pulls the trigger?

Oxidative protein modifications in diabetes and neurodegenerative diseases

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Reactive oxygen and nitrogen species (RS) are produced in metabolic pathways of aerobic organisms. Although they are counter balanced by antioxidant enzymes and antioxidants, there is always a moderate excess of RS, some of which participate in cell signaling [1]. One possibility of cell signaling is through protein oxidation. Irreversible protein oxidations, including protein carbonylations, are mainly thought to have deleterious effects, although there seem to be exceptions [2]. Reversible protein oxidations are important posttranslational modifications that can protect the target proteins from further oxidation and have a role in redox signalling cascades. These reactions, mainly modifications of cysteine residues, can boost cellular defense systems against stress or induce apoptosis. The examples of reversible protein oxidative modifications include glutathionylation, S-sulfenylation and S-nitrosylation. Protein S-nitrosylation can be induced by NO and nitrosonium cation (NO+) [3]. Not all, only specific cysteine residues within a particular protein are S-nitrosylated. NO can S-nitrosylate caspases, for example, caspase-3 at the active site cysteine [4]. This prevents its function and results in cell survival. In contrast, the S-nitrosylation of an E3 ubiquitin ligase XIAP (X-linked inhibitor of apoptosis), which targets caspases for degradation, including the caspase-3, results in its inactivation and increased caspase activity. XIAP can be S-nitrosylated by transnitrosylation from S-nitrosylated caspase3, which results in caspase activation and can contribute to neuron damage and neurodegenerative disease. Its role in diabetes development will have to be confirmed. While many different proteins are reported to be S-nitrosylated either in Alzheimer's disease (AD) or in diabetes [5, 6], only a few S-nitrosylated proteins are known to have a role in the pathology of both diseases. One of them is the insulin degrading enzyme (IDE). The S-nitrosylation of IDE is linked to the increased insulin levels that contribute to insulin resistance in diabetes mellitus type 2 [7]. IDE is also involved in amyloid beta degradation. Recently it was demonstrated that high glucose and amyloid beta oligomers coordinately produce an increase of reactive nitrogen species that suffice for increased S-nitrosylation of IDE in rodent and human cells [8]. Complex combinations of several S-nitrosylated residues in IDE can result in the protection of enzyme activity or in IDE inactivation, conformational change, aggregation and therefore loss of its activity [5]. Thus the decreased activity of IDE can contribute to the pathology of diabetes and AD; an interesting find, since diabetes mellitus type 2 is a risk factor for AD.

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Gene environment interactions: the case of asbestosis

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Introduction: It has become increasingly obvious that both environmental and genetic factors may influence the development of many diseases. Primary candidates for gene-environment interactions studies have been mostly genes coding for enzymes involved in the detoxification processes. Several studies indicate that reactive oxygen and nitric species (ROS and RNS) may be generated by asbestos fibres. Human tissues contain specific enzymes that participate in metabolism of ROS and RNS, such as superoxide dismutases (SOD-s), catalase (CAT) glutathione S-transferases (GSTs) and inducible nitric oxide synthase (iNOS). The genes coding for these enzymes are polymorphic. The gene-environment interactions will be presented on the example of our study into asbestosis, which is one of the most frequent asbestos related diseases.

Methods: The nested case-control study included 262 cases with asbestosis and 265 controls with no asbestos-related disease. Data on cumulative asbestos exposure and smoking were available for all subjects. PCR based methods were used to genotype MnSOD Ala −9Val, ECSOD Arg213Gly, CAT −262C>T, iNOS (CCTTT)n, GSTM1-null, GSTT1-null, GSTP1 Ile105Val and Ala114Val polymorphisms. To assess asbestosis risk, logistic regression analysis was used.

Results: The OR of asbestosis was 3.21 (95% CI 2.43−4.23) for cumulative asbestos exposure; 0.98 (95% CI 0.69−1.39) for smoking; 1.50 (95% CI 1.01−2.24) for MnSOD −9Ala/Ala versus Ala/Val and Val/Val; 1.63 (95% CI 0.62−4.27) for ECSOD 213Arg/Gly versus Arg/Arg; 1.36 (95% CI 0.70−2.62) for CAT −262TT versus CT and CC; 1.20 (95% CI 0.85−1.69) for iNOS LL versus SL and SS; 1.01 (95% CI 0.71−1.43) for GSTM1-null; 0.61 (95% CI 0.40−0.94) for GSTT1-null; 1.52 (95% CI 1.08−2.15) for GSTP1 105Ile/Ile versus 105Ile/Val and 105Val/Val; and 0.97 (95% CI 0.64−1.48) for GSTP1 114Ala/Ala versus 114Ala/Val and 114Val/Val. The associations between MnSOD Ala−9Val polymorphism and the risk of asbestosis and between iNOS genotypes and asbestosis were modified by CAT −262 C > T polymorphism (p = 0.038; p = 0.031). A strong interaction was found between GSTM1-null polymorphism and smoking (p = 0.007), iNOS (CCTTT)n polymorphism and smoking (p = 0.054), and between iNOS (CCTTT)n polymorphism and cumulative asbestos exposure (p = 0.037).

Conclusions: The findings of our study into asbestosis and the results of other studies suggest that in addition to environmental and/or occupational exposure to different hazards and lifestyle factors, the genetic factors as well as the interactions between different genotypes, genotypes and lifestyle factors, and between genotypes and environmental/occupational exposure to hazards have an important influence on the development of diseases and should be further investigated.

Rare genetic variants do play a major role for interindividual variation in drug response

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We characterized the genetic variability of 146 clinically relevant genes influencing drug pharmacokinetics in African and European subpopulations, which pose key determinants for inter-individual variations in drug efficacy and adverse drug reactions. By integrating data from the 1000 Genome (n=1092 individuals) and the Exome Sequencing projects (n=6503 individuals), single nucleotide variants (SNVs) were identified and analyzed regarding frequency, functional consequences and ethnic diversity. In total, we found 12,152 SNVs in exons, 312 of which were novel. The majority of variants was rare (MAF<1%; 92.9%) and non-synonymous (56.2%). We calculate that individuals of European and African descent harbor on average 100.8 and 121.4 variants across the 146 pharmacogenes studied, respectively. Additionally, by analyzing variation patterns across these populations we pinpoint at potential priority genes for population-adjusted genetic profiling strategies. Furthermore, we estimate based on our variant frequency analyses that around 30-40% of functional variability in pharmacogenes is attributed to rare variants. Our results indicate that these clinically important genes are genetically highly variable and differ considerably between populations. Furthermore, the large extent of rare variants emphasizes the need for sequencing-based approaches and effective functionality predictions to allow for true personalized medicine.

Network Data Integration Enables Precision Medicine

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We are faced with a flood of molecular and clinical data. Various biomolecules interact in a cell to perform biological function, forming large, complex systems. Large amounts of patient-specific datasets are available, providing complementary information on the same disease type.

The challenge is how to mine these complex data systems to answer fundamental questions, gain new insight into diseases and improve therapeutics. Just as computational approaches for analyzing genetic sequence data have revolutionized biological understanding, the expectation is that analyses of networked "omics" and clinical data will have similar ground-breaking impacts. However, dealing with these data is nontrivial, since many questions we ask about them fall into the category of computationally intractable problems, necessitating the development of heuristic methods for finding approximate solutions.

We develop methods for extracting new biomedical knowledge from the wiring patterns of large networked biomedical data, linking network wiring patterns with function and translating the information hidden in the wiring patterns into everyday language. We introduce a versatile data fusion (integration) framework that can effectively integrate somatic mutation data, molecular interactions and drug chemical data to address three key challenges in cancer research: stratification of patients into groups having different clinical outcomes, prediction of driver genes whose mutations trigger the onset and development of cancers, and re-purposing of drugs for treating particular cancer patient groups. Our new methods stem from network science approaches coupled with graph-regularised non-negative matrix tri-factorization, a machine learning technique for co-clustering heterogeneous datasets.

Introduction to Multiscale Simulation of Enzyme Catalysis: Application to Monoamine Oxidase Catalyzed Decomposition of Biogenic Amines

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Monoamine oxidase (MAO), which exists in two isozymic forms, MAO A and MAO B, is an important flavoenzyme responsible for the metabolism of biogenic amines such as dopamine, serotonin and noradrenaline. I will present atomic details of the rate-limiting step of dopamine degradation by MAO B, which consists of the hydride transfer from the methylene group of the substrate to the flavin moiety of the enzyme. This contribution builds on our previous quantum chemical study of the same reaction using a cluster model [1], but now considering the full dimensionality of the hydrated enzyme. Well converged activation free energies were calculated by employing the empirical valence bond (EVB) approach of Warshel and coworkers [2]. We show that the MAO B enzyme is specifically tuned to catalyze the hydride transfer step from the substrate to the FAD prosthetic group and that it lowers the activation barrier by 12.1 kcal/mol compared to the same reaction in aqueous solution, giving rise to the rate enhancement of more than 8 orders of magnitude [3]. The calculated barrier in the enzyme of 16.1 kcal/mol is in excellent agreement with the experimental value of 16.5 kcal/mol. Relevance of nuclear quantum effects for this enzyme will be discussed [4,5]. Preliminary results for simulation of MAO A catalyzed decomposition of noradrenaline will be given [5,6]. The effects of point mutations will be presented. The relevance of MAO irreversible inhibition for neurodegeneration will be discussed [7].

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One man's *1 is another man's *13? Trouble with current nomenclatures in personalized medicine

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Introduction: Many currently available pharmacogenomic assays and algorithms interrogate only a small set of 'tag' polymorphisms for inferring haplotypes. In order to estimate the accuracy of such haplotype inferences from limited SNP information (Figure 1) across different populations, we simulated haplotype inferences made by existing pharmacogenomic assays for seven important pharmacogenes based on full-genome data of 2504 persons in the 1000 Genomes dataset.

Results: A sizable fraction of samples did not match any of the haplotypes in the star allele nomenclature systems. We found no clear population bias in the accuracy of results of simulated assays. Detailed results are available in [1].

Conclusions: Haplotype nomenclatures and inference algorithms need to be improved to adequately capture pharmacogenomic diversity in human populations.

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Polymorphism constraint

Haplotype constraint	rs1	rs2	rs3	rs4
GENE*1	A	A	A	A
GENE*2	C	A	A	A
GENE*3	A	C	A	A
GENE*4	C	A	A	C
GENE*5	A	C	C	C
Example 1 → non-problematic *2 call	C	A	A	A
Example 2 → no-call	A	C	C	C
Example 3 → problematic *2 call	C	A	A	C
Example 4 → problematic *3 call	A	C	A	C

Figure 1: Illustration of haplotype inference and potential errors based on limited SNP data.

Emerging technologies in pharmacogenomics

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Advances in personalized medicine have led to an increase in pharmacogenomics studies that involve testing individuals for drug metabolism enzyme and transporter gene polymorphisms implicated in drug response. As a consequence, there is a growing demand for affordable, easy to use technologies with fast sample-to-results workflows that can accommodate testing customizable sets of target gene variants and a changeable number of samples. Additionally, data analysis tools are needed to facilitate translation of an individual's genetic information to their diploid content of gene-level star allele haplotypes, which can be correlated with drug metabolism enzyme phenotypes. We will present the development of a comprehensive pharmacogenomics experiments workflow solution to meet this need. High quality data is generated from purified buccal swab DNAs run with TaqMan® SNP genotyping and copy number assays in OpenArray® and 384-well plate formats, respectively, on the QuantStudio™ 12K Flex system. Data analysis is accomplished using TaqMan® Genotyper™ Software to examine SNP genotyping assay results and CopyCaller® Software to examine copy number assay results, followed by translation of this genetic data for individual samples to star allele genotypes using the recently developed AlleleTyper™ Software. The specific TaqMan® SNP Genotyping and Copy Number Assays to gene variants used can be tailored to suit the needs of a given pharmacogenomics study. This low cost, high throughput pharmacogenomics workflow can be completed in a single day, from sample preparation to data analysis.

Cancer genomics

Clinical implementation of host pharmacogenomics: focus on colorectal cancer

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Colorectal cancer represents one of the most common malignancies and cause of cancer-related deaths worldwide. Despite the multi-agents treatment strategies, including standard chemotherapeutics and novel targeted drugs, produced in the last years a great improvement in the response rate and patient's survival, a remarkable inter-individual variability in therapy outcome still represent a challenging problem for CRC management. Great research efforts have been focused on elucidating the contribution of the host genetic variability on the outcome of backbone chemotherapy agents in colorectal cancer such as fluoropyrimidines (FL), irinotecan (IRI) and oxaliplatin (OXA). We have demonstrated a primary role of UGT1A1*28 polymorphism in the toxicity of IRI based treatment, and developed this marker in the clinical setting by re-defining its maximum tolerated dose in genotype-based phase 1b studies in advanced colorectal cancer. We have also validated the predictive role of DPYD-rs3918290, -rs55886062, and -rs67376798 in the development of severe/ lethal toxicity after FL treatment in a large group of 603 cancer patients from the common clinical practice. More exploratory studies are ongoing investigating the predictive/ prognostic role of panels of SNPs related to FL, IRI and OXA ADME (cellular transporters, nuclear receptors, and oxidative metabolism) and more recently to patients immunological profile. Based on our data and current pharmacogenetic guidelines, we have set up a clinical pharmacogenetic service in CRO-Aviano- Experimental and Clinical Pharmacology Unit. A pharmacogenetic electronic report is embedded in the patient clinical record and a drug dose adjustment is suggested for patients carrying UGT1A1 and/or DPYD risk variants. Among the effects of this experimental service there was a progressive sensitization of the oncologists on pharmacogenetics, demonstrated by the increasing rate by time of patients referred for pre-treatment genotyping. Cost-effectiveness and HTA studies are ongoing to assess the clinical utility of a pre-emptive pharmacogenetic approach in oncology.

Molecular epidemiology of pancreatic cancer

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As the complexity of the biological mechanisms underlying disease increases, so does the need to adopt an interdisciplinary or trans-disciplinary approach in order to disentangle the disease risk factors. For most complex diseases, including pancreatic cancer, the approaches taken are too simplistic to achieve a sufficient understanding of their pathophysiology. In most cases, a context of interactions between multiple genomic, epigenomic and environmental risk factors is involved. Two areas that are related and likely play an important role in cancer are chronic inflammation and the microbiome. In this regard, the challenges posed by pancreatic cancer - and its strong relationship with chronic inflammation - offer a unique opportunity to explore its intrincate aetiological scenario through omics approaches by integrating lifestyle risk factors as well as co-morbidities with biomarkers. We focus on the role of chronic inflammation, both local and systemic, as a major pathophysiological mechanism involved in pancreatic cancer. We build on previous evidence on the importance of this component in cancer development - in general - and the increasing knowledge generated in relationship to pancreatic cancer. Pancreatitis is the most prevalent disorder of the exocrine pancreas. Chronic pancreatitis is an established risk factor for pancreatic cancer. Other systemic inflammatory conditions (i.e. allergies, asthma, body mass index (BMI), type 2 diabetes (T2D), periodontitis, vitamin D levels, and genetic components (ABO and NR5A2 variants, among others)) also influence pancreatic cancer risk pointing to a highly correlated multifactor aetiology. The causal mechanisms behind these associations are unknown. These data underscore the importance of an integrative analysis of the genomic and non-genomic factors associated with the development of pancreatic cancer. An improved understanding of the mechanisms behind chronic pancreatitis, immune response, and pancreatic cancer may lead to novel preventive, diagnostic, and therapeutic strategies.

Electroporation based treatments, electrochemotherapy and electrogene therapy for treatment of cancer

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Electroporation or electropermeabilization is known as a tool for drug and gene delivery into the tumors. The first preclinical studies demonstrated that exposure of tumors to electric pulses can increase the uptake of chemotherapeutic drugs; predominantly of those that have hampered or limited diffusion or carrier mediated transport into the cells. In vitro studies have shown that bleomycin (BLM) or cisplatin (CDDP) uptake into the cells can be increased from 1000 to several 10 folds, respectively. The same was demonstrated in vivo, up to two fold increase of the drug uptake was demonstrated in the tumors, either in the whole tumors, or the drugs bound to DNA. These first results encouraged the field to proceed with the investigations of the appropriate electrical parameters for the efficient electroporation and also the optimal drug dosage as well as timing of the drug injection according to the application of electric pulses; either after intravenous or intratumoral drug injection. The preclinical studies were translated into the clinic, both in human and veterinary medicine. The parameters elaborated in preclinical studies were demonstrated to be effective also in treatment of melanoma, sarcoma and carcinoma tumors. Treated were cutaneous and subcutaneous tumors, with approximately 80% success rate, and approximately 70% complete responses lasting also up to several years. Currently electrochemotherapy is being introduced also for treatment of bigger cutaneous tumors and for treatment of deep seated tumors either in subcutis, bones or in the liver. Another application of electroporation is introduction of nucleic acids into the tissues; like tumors, muscle or skin. The introduction of nucleic acids, due to their size and negative charge is more difficult. The mechanism is not fully understood, however currently it is known that for specific tissue specific electrical parameters are needed. Besides in oncology, electroporation for gene delivery can be used in vaccination and treatment of other genetically based diseases. Electrogene therapy in oncology is also in the clinical stage of testing. It has been used in treatment of tumors of small animas like dogs and also horses, with significant effect demonstrating the feasibility and effectiveness of this treatment. However, the treatment has been translated also into the human clinic; the latest report by Daud et al. 2009 has demonstrated that treatment of melanoma tumor nodules in patients that have progressive disease is effective and the patients have significant benefit of it. In Slovenia we also initiated the first gene therapy trial on melanoma patients that will use antiangiogenic gene coding plasmid and we are in preparation for the IL-12 gene therapy trial. Based on these data is to foresee that electroporation as a physical method of drug and gene delivery is promising and with further development may find the way in its broader clinical practice.

Immunogene therapy for canine cancer: a translational model for human oncology

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Electrogene therapy refers to the introduction of genetic material into the cells or tissues with electroporation for therapeutic purposes. When a therapeutic gene is encoding a protein with immunological properties the therapy is called immungene therapy. In veterinary oncology, the most advanced immunogene therapy is with interleukin-12 (IL-12) alone or in combination with electrochemotherapy, which is another biomedical application of electroporation for treatment of cancer in combination with chemotherapeutic drugs.

Clinical studies in veterinary oncology can represent a bridge between experimental therapies in mice and their translation into human clinical oncology studies. Spontaneous tumors in dogs are a very good model for human tumors, as they share many epidemiological, biological and clinical features with human tumors. Therefore, the studies in canine patients are increasingly more important for development and implementation of new therapies into human clinical trials. In line with that, the demonstration of efficacy, feasibility and safety of immunogene therapies alone or in combination with standard treatments in canine patients could support further development and evaluation of these therapies in human clinical trials.

In veterinary oncology, gene therapy studies of IL-12 mostly used human or feline IL-12 due to the high homology between canine and the mentioned cytokines and the non-availability of canine IL-12. Immunogene therapy with IL-12 was tested in several different tumor types in dogs. The routes of administration were either intramuscular, intratumoral or peritumoral. The intratumoral therapy resulted in better antitumor effectiveness compared to intramuscular electrogene therapy. Nevertheless, longer survival of the dogs treated with immunogene therapy was obtained compared to survival of dogs treated with standard treatments. Combination of immunogene therapy with IL-12 and electrochemotherapy was performed with both drugs used in electrochemotherapy; bleomycin and cisplatin. In this case, plasmid encoding IL-12 was injected peritumorally. Combined therapy proved to be safe, resulting in complete responses of different tumor types in dogs. First studies with IL-12 immunogene therapy have already started in human oncology, therefore our studies in canine patients form the basis to translate the combined treatment approach also to human oncology.

A comprehensive view of Liquid Biopsy from ThermoFisher Scientific

Christopher M. Allen
Thermo Fisher Scientific

Liquid Biopsies are providing us with the unique opportunity to not only look at cancer diagnosis in a non-invasive way but also to engage in longitudinal studies and adjust therapies accordingly. Liquid Biopsies are utilising a range of techniques for analysis and different template types and Thermo Fisher is committed to providing various tools to perform these analyses.

Role of pharmacogenetics in metastatic breast cancer (MBC) patients treated with exemestane as first-line hormone therapy. An Italian multicentre study.

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Introduction: Anti-aromatase therapy represents the treatment of choice of hormone-receptor (HR)−positive metastatic breast cancer (MBC). Exemestane is a third generation aromatase inactivator widely used in the treatment of advanced BC, but unfortunately, in a subset of patients it is not effective. For this reason there is a compelling need of finding predictive and prognostic biomarkers of exemestane efficacy. Single Nucleotide Polymorphisms (SNPs) of genes involved in estrogen availability, in pharmacokinetics (PK) and pharmacodynamics (PD) of exemestane could be responsible for the lack of response. The aims of this study were:

- To assess the prognostic value of polymorphisms related to hormone metabolism/drug PD an PK on 5 year progression free survival (PFS) and 5 year overall survival (OS);
- To assess the role of such polymorphisms on the response rate (RR) to exemestane therapy.

Results: For this multicenter study, 423 metastatic or locally advanced BC patients treated with exemestane as a first-line hormone therapy were prospectively enrolled from 2007 to 2012. Among them, 302 patients with blood sample for DNA extraction and complete clinical data were included. Patients were genotyped for 15 polymorphisms in genes related to hormone metabolism/exemestane PD and PK. Genetic data were associated with clinical outcome by adjusted logistic and cox regressions. RR was defined as patients with a complete response (CR) or a partial response (PR) as their best clinical response obtained.

The SNP CYP19A1 1558 T/C (rs10046) on the aromatase gene, was associated to 5 year PFS (Adj HR=1.15, 95%CI =1.03-1.30, p=0.035) and showed a trend for association with 5 years OS (Adj HR=1.14, 95%CI =0.97-1.34, p=0.113).

ESR2 1730 A/G (rs4986938) and of UGT1A1*28 were associated to 5 year OS according to a recessive and a dominant model, respectively (ESR2 1730 A/G: Adj HR=1.47, 95%CI =1.06-2.03, p=0.020; UGT1A1*28: Adj HR=1.5, 95%CI = 1.15-1.97, p=0.003).

Regarding the RR, the SNP CYP19A1 410 A/C (rs4646) was predictive of a better RR according to the additive model (Adj OR= 1.50, 95%CI =1.17-2.67, p=0.002), as well as the SNP RIZ1 delP704 (rs2308040) which was associated to RR according to the recessive model (Adj OR = 2.23, 95%CI =1.25-4.00, p=0.007).

Conclusions: This study pointed out five potential predictive or prognostic genetic biomarkers, which, once validated, could be employed in the daily clinical practice to personalize exemestane treatment.

Acknowledgments: Experimental and Clinical Pharmacology Unit − CRO Aviano, Dr. Giuseppe Toffoli and all the participating centers.

Clinical-pharmacogenetic model predicting treatment outcome in malignant mesothelioma

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Introduction: Malignant mesothelioma (MM) is an aggressive cancer with poor prognosis. Most patients are treated with gemcitabine/cisplatin or pemetrexed/cisplatin chemotherapy, but there are large interindividual differences in treatment outcome. As both clinical characteristics and genetic variability may affect treatment outcome, our aim was to construct and validate clinical-pharmacogenetic prediction models of outcome of gemcitabine/cisplatin or pemetrexed/cisplatin treatment and to develop an algorithm for genotype-based treatment recommendations.

Patients and methods: In total, 169 MM patients were included in our study. 71 gemcitabine/cisplatin-treated and 57 pemetrexed/cisplatin-treated MM patients were included in the training groups used to build the respective clinical-pharmacogenetic models. Pharmacogenetic scores were assigned by rounding the regression coefficients. Gemcitabine/cisplatin model was validated on 66 independent MM patients.

Results: Clinical-pharmacogenetic model predicting outcome of gemcitabine/cisplatin included CRP level, histological type, performance status, RRM1 rs1042927, ERCC2 rs13181, ERCC1 rs3212986, and XRCC1 rs25487 and had values ranging between 0 and 3.4. Cutoff value of 0.75 had sensitivity of 0.62 and specificity of 0.81. Patients with higher score had shorter progression-free survival (PFS) ($P<0.001$) and shorter overall survival (OS) ($P<0.001$). In the validation group, positive predictive value was 0.74 and negative predictive value was 0.56. Clinical-pharmacogenetic model predicting outcome of pemetrexed/cisplatin included CRP level, MTHFD1 rs2236225, and ABCC2 rs2273697 with scores ranging between 0 and 3.9. Cutoff value of 2.7 had sensitivity of 0.75 and specificity of 0.61. Patients with higher score had lower probability of good response and shorter PFS ($P<0.001$).

Conclusions: Clinical-pharmacogenetic models could enable stratification of MM patients based on their probability of response to gemcitabine/cisplatin or pemetrexed/cisplatin and improve treatment outcome. This approach could be used for translation of pharmacogenetic testing to personalization of chemotherapy as a way of selecting the most favorable treatment option for each patient.

Pharmacogenomic and epigenomic biomarkers for prediction of drug response

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There are pronounced interindividual variations in drug metabolism, drug response and incidence of adverse drug reactions. In addition to genetic variation, epigenetic and long non-coding (lncRNA) dependent regulation of these genes is important and future direction in this novel research field is outlined with respect to our understanding of interindividual differences in drug action (Ivanov et al., 2012). A novel class of drugs, so called epidrugs, are known to can intervene in the epigenetic control of gene expression for disease treatment, and many so called epidrugs are now in clinical development. In addition, disease diagnosis prognosis and drug treatment success can be monitored by epigenetic biomarkers. Regarding the genetic variation it is clear that in addition to common previously characterized variations of importance for drug response which are frequently utilized for current therapy, there are also a huge number of rare gene variants of importance for the individual response worth attention. Indeed studies in monozygotic and dizygotic twins as well as analyses of large whole genome and whole exome sequencing projects reveal that only about 50-70 % of the true interindividual variation in drug pharmacokinetics can be assigned to known mutations commonly analyzed for. The lecture will give an update in the field of current and future genomic biomarkers, epigenomic alterations during development and epigenetic mechanisms of importance for prediction of drug metabolism, drug action and ADRs focusing on the most clinically relevant examples.

EUPancreas - An integrated European platform for pancreas cancer research: from basic science to clinical and public health interventions

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Pancreatic cancer (PC) has the lowest survival rate of any cancer. Moreover, death rates from PC are rising across Europe while those from all other cancers continue to fall. PC research is urgently needed though it poses several challenges requiring sustained multidisciplinary collaborative efforts. To gain knowledge on PC and to lower the burden on society, it was deemed necessary to develop a comprehensive pancreatic research community, to provide the tools and resources needed in order to make scientific breakthroughs. The EUPancreas COST Action (BM1204) represents a unique European platform that facilitates the collaboration of a broad range of European and international PC multidisciplinary research groups. It brings together a group of young researchers across a range of disciplines in collaboration with more experienced researchers and allows Europe to actively participate in the international scenario of PC research. Overall, the Action involves >200 multidisciplinary members from 22 EU countries. The main objective of EUPancreas is to capitalise on emerging scientific and technological developments in the field of PC, in order to integrate knowledge and experience in a multidisciplinary way "from cell to society". This objective is pursued through the promotion and application of uniform study tools and protocols, fostering their optimal use by early-stage researchers, enhancing the mobility and training of researchers (through Short-Term Scientific Missions, training schools, workshops, etc.), and disseminating the results produced by the Action to the broader society. EUPancreas builds together with other international initiatives to increase public and health policy-maker awareness about PC research needs and impacts. EUPancreas is itself divided into 4 Working Groups, each of which leads a thematic area: 1) Harmonization of research tools, with regard to standardization of PC definitions and development of protocols for the collection of biological samples and epidemiological/clinical information; 2) Integration of omics data within and between studies, by evaluating existing omics data and establishing approaches for data deposit; 3) Translation of findings into clinical practice, through the design of optimal studies to address clinical research questions and biomarkers research in PC; and 4) Pancreatic cancer patient management, aimed at developing evidence-based PC-best practice guidelines and to translate them into healthcare systems in Europe. EUPancreas is thereby developing novel interdisciplinary utensils and methods to improve our understanding of PC and its control by answering questions related to its aetiology, early detection, and evidence based personalized treatment to enhance primary, secondary, and tertiary prevention, as well as on health management.

Genotype-based dosing of oral anticoagulants in clinical practice

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Anticoagulants are widely prescribed for the treatment and prevention of cardiovascular diseases. Coumarin anticoagulants are characterized by narrow therapeutic index and inter-individual or intra-individual variability in response to the treatment. This variability can be explained by clinical factors such as age, sex, and drug-drug interactions, but also by genetic variants. Relevant polymorphisms related to anticoagulants have included genes that participate in the drugs' pharmacokinetics and target genes. Observational studies have indicated potential benefits of CYP2C9 and VKORC1 guided dosing of coumarin anticoagulants but randomized clinical trials resulted in mixed results. Prospective trials suggest that incorporation of genotype results in faster time to therapeutic range than without; however, whether these improvements result in improved clinical outcomes is still unclear. Since clinical evidence for pharmacogenetics-guided warfarin dosing is rather limited to intermediary outcomes, researchers need to determine the precise impact of genotype-guided warfarin therapy on patient outcomes. New oral anticoagulant (NOACs) agents are now being used which specifically inhibit either thrombin (dabigatran) or factor Xa (rivaroxaban, apixaban). Unlike coumarin derivatives, NOACs have a wide therapeutic index, a rapid onset of action and were supposed to not require routine laboratory monitoring. Since postmarketing surveillance pointed to the bleeding complications in some patients reliable biological predictors could help to optimize treatment and pharmacogenetics may play a role in NOACs treatment. Bioavailability of dabigatran etexilate, rivaroxaban, and apixaban is dependent on the membrane transporter P-glycoprotein (P-gp), which is also modulated by a variety of drugs and food components. ABCB1 gene coding for P-gp is polymorphic and it could have pharmacogenetic relevance. GWAS undertaken as part of the RE-LY Trial in patients with atrial fibrillation identified genetic determinants in ABCB1 and CES1 loci that were associated with a significant decrease in dabigatran concentration and a lower risk of bleeding. Differential metabolism by CYP3A4 enzyme may have an influence on rivaroxaban pharmacokinetics, and recently discovered polymorphisms in CYP3A4 gene may contribute to variability in rivaroxaban metabolism as well to drug-drug interactions. Researchers continue to determine the precise impact of genotype-guided warfarin therapy on patient outcomes. Ongoing trials will provide a clearer picture of whether genotype-based warfarin dosing improves outcomes and may, therefore, subsequently be compared with the NOACs. Although some published data indicate that pharmacogenomics-guided warfarin treatment represents a cost-effective therapy, there is still a lack of sufficient information on the cost-effectiveness of anticoagulation treatment options.

Preemptive testing in pharmacogenomics

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The translation from empirical population-based treatment approaches to preventive, personalized, predicitive and participatory health care enabled by genomic medicine was enabled by developing evidence based recommendations and guidelines on how to use genetic test results in specific drugs, genes and genotype/phenotype. Such guidelines were initially developed by Clinical Pharamcogenomics Implementation Consortium (CPIC) for 10 genes and 24 drugs (https://www.pharmgkb.org/page/cpic). The list of gene-drug pairs has been extended to 53 drugs and 11 genes by Dutch Pharmacogenetics Working Group (DPWG; https://www.pharmgkb.org/page/dpwg). The CPIC and DPWG guidelines were made to support the decision whether to avoid or modify a dose of a particular drug in patient with genotype at risk.

The adoption of these guidelines into health care system was limited because this approach requires that data on patients genetic characteristics that may effect treatment are available before the decision on which drug to prescribe is made. When pharmacogenetic genotyping was first employed in clincial practice, patients were mostly genotyped only for the gene actionable for a particular high risk drug for which therapeutic recommendations were available

This differs from the preemptive testing approach, which is based on prospective genotyping for multiple pharmacogenes with available therapeutic recommendations and storing the data in such a way that the relevant pharmacogenetic information and the accompanying alerts and treatment recommendations are available to the physician and/or pharmacist when prescribing/ dispensing the drug to the patient. Such approach has been sucessfully implemented in five USA centers (1-5) and is currently being launched in Europe with the Horizon2020 project Ubiquitous Pharmacogenomics (U-PGx): Making actionable pharmacogenomic data and effective treatment optimization accessible to every European citizen (http://upgx.eu/). This is the first initiative to implement a panel-based, pre-emptive PGx strategy and integrate this into routine clinical practice across seven European countries: The Netherlands, Spain, Austria, Italy, Greece, Slovenia and UK.

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Bridging theory and practice: Clinical decision support systems for personalized medicine

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Research in personalized medicine and pharmacogenomics has made significant progress and a wide variety of clinical guidelines for utilizing genetic information for optimizing drug treatment has become available. However, it remains difficult for clinical practitioners who are not experts in pharmacogenomics to adopt these insights as part of their daily routine.

In this talk I will give an overview of how computers and clinical decision support tools could enable medical professionals (and patients) to make use of pharmacogenomic findings at the point-of-care. I will discuss general principles that are important for successful implementation of decision support systems, and will also discuss specific examples, such as the mobile Mediction Safety Code system we have developed (Figure 1) [1].

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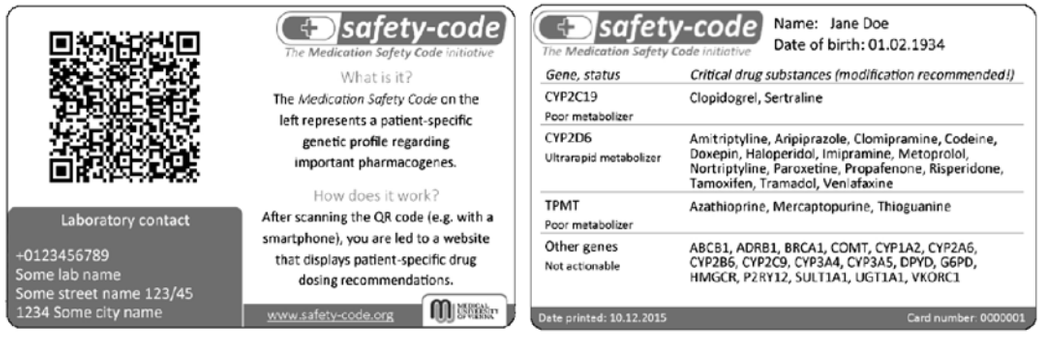

Figure 1: A prototype of a pocket card for making pharmacogenomic data and decision support available at the point-of-care.

Closing plenary lecture

Genomics and Therapeutics: The Opportunities

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A key issue for the genomic revolution is how it will help in improving treatment for diseases. As the number of people globally who have their genomes sequenced increases, the depth and breadth of information available will allow us to progress personalised or precision medicine, to ensure that patients get the right treatment at the right dose and at the right time. This will be crucial in ensuring that we optimise the benefit-risk ratio of all therapies (new and old) maximizing efficacy and minimizing safety. In some cases, genomic information will be directly relevant to the development of new therapies – this is an appropriate strategy given that drugs which are developed on the basis of genetic information seem to have a higher success rate to go to market. Genomic information can also provide insight which may lead to re-purposing of medicines for diseases for which there is unmet medical need. Genomic information may also be important in improving the use of drugs which have been available over many years to (a) change the intensity of therapy; and (b) improve the benefit-risk profile. Many of these areas will be covered in the lecture, with appropriate examples. In summary, the genomic revolution offers many opportunities for therapeutics – the key issue will be to develop the evidence base which allows the genomic findings to be translated into patient care.

P01

Molecular characterization of genetic variants in patients with primary ciliary dyskinesia from Serbia

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Introduction: Primary ciliary dyskinesia (PCD) is a rare, autosomal recessive disorder with extensive genetic heterogeneity and is estimated to affect 1 in 15,000 to 30,000 individuals. It usually comes to medical attention because of recurrent pneumonia, otitis media and upper respiratory tract infections early in the life, or, in the adult male, infertility. Respiratory infections are caused by defective mucociliary clearance due to immotile or dysmotile respiratory cilia with or without ultrastructural defects.

Results: In this study, we analyzed 6 patients from Serbia with diagnosis of PCD according to clinical presentation. We used a NGS panel with 4813 genes to detect disease-causing mutations in these patients. We have detected variants in 6 different genes. In each patient only one gene was affected. Variants were detected in the genes previously associated with PCD: DNAL1 (c.347A>T, c.350T>G and c.485G>A), LRRC6 (c.27T>G and 1397T>C), DNAH11 (c.7798C>T, c.8555C>G), DNAH5 (c.1137C>T, c.7624T>C, c.4356-2A>G) and CCDC40 (c. [248delC]; [248delC]), from first to the fifth patient, respectively. In the last patient we detected homozygous variant in SCNN1A gene (c. [1654T>C]; [1654T>C]). So far, SCNN1A gene was not associated with PCD, but it was associated with Cystic Fibrosis-Like Disease.

Conclusions: This study provided the first data about molecular genetics of patients from Serbia presenting with PCD clinical symptoms, thus paving a path to molecular genetic diagnostics and genetic counseling of this disease in the country.

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P02

Nucleosides blunt effects of AMPK activator AICAR in cultured skeletal muscle and cancer cells

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Introduction: AMP-activated protein kinase (AMPK), an energy-sensing kinase that regulates cell metabolism, proliferation, and apoptosis, is a major target for development of novel anti-diabetic and anti-cancer treatments. AICAR is the most widely used pharmacological AMPK activator and is commonly used as a standard to which novel AMPK activators are compared. However, the extent of AICAR-stimulated AMPK activation varies across cell types and experimental conditions. As an adenosine analogue, AICAR is taken up via nucleoside transporters and, once inside the cell, enters purine metabolic pathways. Nucleosides, present in standard cell culture media, might therefore modulate AICAR uptake or metabolism. We investigated whether presence or absence of nucleosides alters effectiveness of AICAR as an AMPK activator.

Results: To examine whether nucleosides alter AICAR-stimulated AMPK activation, cultured L6 and human skeletal muscle cells were incubated in MEMĐ with or without nucleosides. In MEMĐ with nucleosides AICAR failed to stimulate phosphorylation of AMPK (Thr172) and its downstream target acetyl-CoA carboxylase (ACC) (Ser79). Conversely, in MEMĐ without nucleosides AICAR markedly increased phosphorylation of AMPK and ACC. AICAR also stimulated AMPK activation in skeletal muscle cells if they were grown in nucleoside-free DMEM. Similarly, AICAR stimulated AMPK and ACC phosphorylation in MDA-MB-231 breast cancer cells if they were incubated in RPMI1640, which does not contain nucleosides, but not if they were incubated in MEMĐ with nucleosides. In contrast, PC-3 prostate cancer cells were responsive to AICAR even in MEMĐ with nucleosides. Nevertheless, nucleosides blunted AICAR-induced arrest of cell proliferation in MDA-MB-231 and PC-3 cancer cells. Besides nucleosides, MEMĐ contains several other components which might affect AMPK activation by AICAR, including vitamin B12, vitamin C and lipoic acid. However, we show that these components cannot explain profound differences in AICAR-stimulated AMPK activation in different media. We also show that the expression of AICAR transporters (equilibrative nucleoside transporters 1 and 2) as well as AICAR-metabolizing enzymes (adenosine kinase and inosine monophosphate 5-aminoimidazole-4-carboxamide ribonucleotide transformylase/inosine monophosphate cyclohydrolase) in L6 skeletal muscle cells was similar in the presence or absence of nucleosides.

Conclusions: Together, our results show that nucleosides in cell culture media blunt the efficiency of AICAR as an AMPK activator and an anti-proliferative agent. Our results indirectly suggest that nucleosides in cell culture media competitively inhibit the cellular uptake of AICAR and thus blunt its intracellular effects. These findings highlight media composition as an important source of experimental variation in cell-based assays for identification of novel AMPK activators.

P03

Genetic markers in Alzheimer's disease

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Introduction: Alzheimer's disease (AD) is a progressive neurodegenerative disorder and mechanisms that fully explain its pathogenesis are still not entirely elucidated. Within the preparative phase of the ARTEMIDA project we reviewed the current knowledge on the genetics of AD, in particular the candidate genetic markers of the disease risk and prognosis that could be used in the clinical practice.

Results: Early onset AD (EOAD) is mostly caused by three genes with autosomal dominant inheritance: APP, PSEN1, and PSEN2. Late onset AD (LOAD) is on the other hand a multifactorial condition, where a combination of genetic, lifestyle and environmental factors contribute to its development. However, it is estimated that as much as 60-80 % of LOAD risk is inherited [1]. Multiple candidate genes studies and genome-wide association studies (GWAS) have identified genetic markers associated with LOAD. APOE is the major risk gene identified. All other identified genes have much smaller effect sizes on AD risk. According to Holmans' pathway analysis these genes are roughly implicated in four different biological pathways: immunity, cholesterol metabolism, endocytosis and ubiquitination [1]. Recently, GWAS and meta-analyses of GWAS data have largely improved our knowledge about genetic contributions to AD risk. The largest study to date has been performed in 2013 by International Genomics of Alzheimer's Project (IGAP), where 11 single nucleotide polymorphisms (SNPs) were newly confirmed as genetic markers in AD [1]. It is however unlikely those individual SNPs would have a significant and clinically relevant affect on AD risk, although they may reach a significant effect in combination or could be related to a specific heritable phenotype of AD [2]. In light of this, some studies have demonstrated the association of LOAD risk loci with age at onset [2, 3]. There is also increasing interest of identifying genetic markers that would predict progression from mild cognitive impairment (MCI) to AD. Recently clusterin gene (CLU) was identified as a potential marker of MCI to AD progression [4].

Conclusions: The latest advances in the field of genetics of AD provide hope for implementation of genetic markers into daily clinical practice.

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P04

Antioxidant Enzyme Polymorphisms and Sequelae after Neonatal Hypoxic-Ischaemic Brain Injury

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Introduction: Hypoxic-ischaemic perinatal brain injury (HIBI) leads to the formation of reactive oxygen species (ROS) and the resultant cell and tissue damage may cause neurological sequelae such as cerebral palsy and/or epilepsy. A decrease in the capacity for defending against ROS may increase the susceptibility to brain damage and poor neurological outcome. The aim of this study was to investigate the impact of common functional polymorphisms in the antioxidant genes SOD2, GPX1 and CAT, associated with a decreased capacity for defence against ROS, in patients with perinatal hypoxic-ischaemic brain injury (HIBI).

Methods: 230 consecutive patients with epilepsy and/or cerebral palsy (CP) and 95 healthy controls were included. Patients' clinical data were collected retrospectively. Real-time PCR based methods were used to genotype GPX1 rs1050450, SOD2 rs4880 and CAT rs1001179. Statistical analysis using logistic regression was performed.

Results: In 214 patients with epilepsy (64 with neonatal and 150 without HIBI), GPX1, SOD2 and CAT polymorphisms were not associated with the risk for epilepsy as compared to healthy controls. Similarly, the frequency distribution of GPX1, SOD2 and CAT genotypes did not differ significantly when 64 patients with epilepsy after HIBI were compared to healthy controls.
Among 80 patients with HIBI, cerebral palsy was present in 51 patients. Carriers of at least one polymorphic CAT rs1001179 allele had significantly higher risk for cerebral palsy (univariable logistic regression, p = 0.026; OR = 3.36; 95 % CI = 1.16–9.76). This difference remained significant after accounting for prematurity (multivariable logistic regression, P_{adj} = 0.011, OR = 5.15, 95 % CI = 1.47-18.09). No differences were observed in the distributions of GPX1 and SOD2 genotypes (p = 0.816 and p = 0.588).

Conclusions: Common GPX1, SOD2 and CAT polymorphisms influence neither the overall risk for epilepsy nor the risk for epilepsy after HIBI. CAT rs1001179 is associated with development of cerebral palsy after HIBI.

P05

Plasma of HIV infected patients with undetected viral load contains exosomes with viral protein Nef

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While anti-retroviral therapy (ART) has improved and saved the lives of people living with HIV, virus persists in the body and contributes to diverse health problems (importantly neurocognitive disorders). Protein Nef has many proven roles in HIV pathogenesis, as it affects cellular signaling and metabolism and viral infectivity. We further showed that Nef, released with exosomes from HIV-infected primary T cells, causes apoptosis in resting lymphocytes. In the present study we aimed to explore if Nef is present in plasma of HIV infected patients and to define its nature (virus/exosome) and cellular source. To this end we performed Nef-ELISA on 134 plasma samples to determine Nef concentration in 26 uninfected and 108 HIV-infected patients (28 non-controllers, 38 ART controllers, 42 elite controllers) obtained from well-characterized SCOPE collection. Next we performed p24-ELISA, AChE activity and western blot analysis of particles released from HIV (VSV-G pseudotyped NL4-3 and YU2) infected microglia and astrocytes, important HIV reservoirs. Nef was detected in 63 patients: 0 uninfected, 23 non-controllers (Mdn (25-75%) = 11.6 (9.0-13.3) ng/ml), 18 ART controllers (8.3 (6.8-10.9) ng/ml) and 22 elite controllers (8.8 (6.5-11.7) ng/ml). Nef concentration positively correlated with viral load (p = 0.024) only in non-controllers, which implies that the source of plasma Nef in HIV controllers does not origin from viruses in plasma. After infecting microglia and astrocytes with pseudotyped HIV cells released viruses (contain p24) and exosomes (show AcHE activity) with at least a subpopulation (contain flotillin) of both containing Nef. Here we showed that Nef is present in plasma of most HIV non-controllers and half of HIV controllers. We hypothesize that the source of plasma Nef in non-controllers are mostly viruses, whereas in virologic controllers the main source are Nef exosomes, released at least from HIV infected microglia, astrocytes and T lymphocytes.

P06

Analysis of PAI-1 gene 4G/5G polymorphism in patients with rheumatoid arthritis

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Introduction: Rheumatoid arthritis (RA) is a chronic inflammatory disease [1]. It is considered that in RA patients fibrin deposition in the joints contribute to their impairment. Degradation of fibrin is performed by plasmin. Plasminogen activator inhibitor-1 (PAI-1) is the main inhibitor of the process through which plasminogen is converted to plasmin [2]. Polymorphism 4G/5G is located in promoter region of PAI-1 gene, and it has been shown that allele 4G corresponds to elevated expression of PAI-1 gene [3, 4]. The aim of this study was to assess the relationship between 4G/5G PAI-1 polymorphism and clinical manifestations in patients with rheumatoid arthritis.

Methods: We have genotyped 169 RA patients using PCR - RFLP method. Disease activity was estimated using disease activity score in 28 joints (DAS28) based on EULAR criteria. Seropositivity was estimated by presence of rheumatoid factor (RF). Additionally we have compared age of onset of the disease and number of swollen and tender joints among patients with different genotypes.

Results: Observed frequencies of genotypes 4G/4G, 4G/5G and 5G/5G were 33.73%, 35.51% 31.36%, respectively. Patients with different genotypes were not significantly different in number of swollen (p=0.465) and tender joints (p=0.427), nor different in disease activity (DAS 28) at the beginning of the disease (p=0.427). Age at onset of disease was not significantly different with respect to genotype (p=0.478). Seropositivity was not correlated with any PAI-1 genotype (p=0.465).

Conclusions: 4G/5G PAI-1 polymorphism does not affect the activity of rheumatoid arthritis.

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P07

Primary human hepatocyte spheroids as a model system for drug-induced liver injury, liver function and disease

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Primary human hepatocytes (PHH) cultured in 2D monolayers are considered the gold standard to study drug-induced liver injury (DILI), liver function and disease. However, PHH rapidly dedifferentiate in 2D, which restricts their usefulness substantially. Here, we present an easily scalable 3D PHH spheroid system in which the hepatocytes are kept in chemically-defined, serum-free conditions. We found that the proteome of PHH spheroid cultures was similar to the proteome of the liver in vivo and even retained their inter-individual variability. The PHH spheroids remained phenotypically stable and retained morphology, viability, and hepatocyte-specific functions for culture periods of at least five weeks. Furthermore, under chronic exposure, the sensitivity of the hepatocytes towards a set of hepatotoxicants drastically increased. Interestingly, the chronic toxicity of fialuridine was mimicked in the PHH spheroids, which was not possible to detect using conventional in vitro systems. Additionally, we show that liver pathologies such as cholestasis and steatosis can be studied in the PHH spheroids. For example, we found that the PHH spheroids can constitute a model for prediction and studies of drug-induced cholestasis. Co-exposure to a non-toxic bile acid (BA) mixture and cholestatic hepatotoxicants resulted in a synergistic increase in toxicity, whereas no such synergism was observed with non-cholestatic hepatotoxicants. In addition, exposure to cyclosporine A rapidly induced lipid accumulation, which could be prevented by co-treatment with the anti-oxidant Ð-tocopherol, suggesting the possibility to study reversible steatosis in the PHH spheroids. In conclusion, the PHH spheroid system represents a versatile and promising in vitro system to study inter-individual variability in liver function, liver disease and DILI.

P08

The role of in vitro innervated human skeletal muscle cells in neuromuscular research

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Skeletal muscles, the largest body tissue, are essential for locomotion, breathing, posture, speaking as well as non-motor functions, such as glucose and potassium metabolism. In addition, they contain protein reserves, which can be mobilized to support stress response under pathological conditions, including infection and trauma. Neuromuscular disorders (NMDs) can severely impair different aspects of skeletal muscle function, thus increasing morbidity and mortality. Unfortunately, only a few effective treatments currently exist in armamentarium against NMDs.

To uncover new pharmacological targets for treatment of NMDs reliable in vitro models are required. One of the most advanced models for in vitro neuromuscular research is a co-culture of rat embryonic spinal cord and human skeletal muscle cells. This model has all the advantages of standard cell culture, while still being sufficiently complex to study major neuromuscular phenomena. For instance, while aneural human skeletal muscle cells do not develop capacity to contract, innervation induces their further maturation, thus leading to development of functional contractile apparatus and spontaneous contractions in vitro (Figure 1).

In this model we can investigate mechanisms underlying metabolic adaptations, ion transport, myotoxicity, as well as pharmacodynamics of various compounds. More important, translational potential of our results is increased by using contracting, functionally mature, skeletal muscle cells.

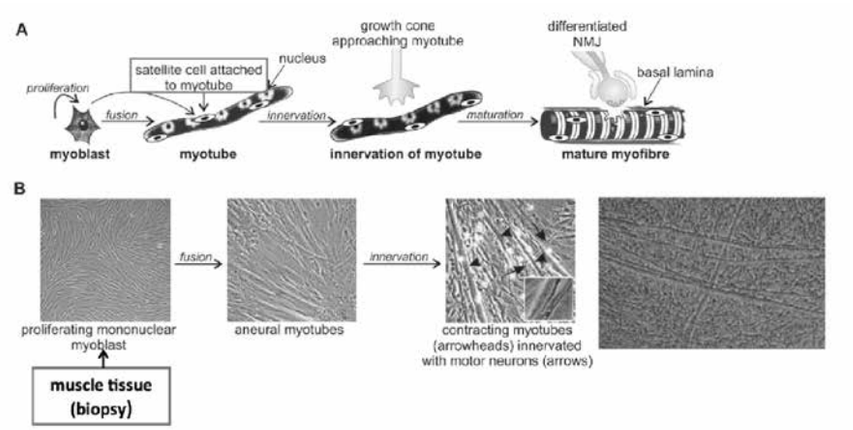

Figure 1: Development of skeletal muscle in vivo (A) and in vitro (B), adopted from Marš T et al. The Effects of Organophosphates in the Early Stages of Human Muscle Regeneration. In: Gupta RC, Handbook of toxicology of chemical warfare agents, 2nd Ed. Academic Press; 2015.

P09

Clinical pharmacogenetic model of response to methotrexate monotherapy in Slovenian and Serbian rheumatoid arthritis patients: differences in patient's management preclude generalization of the models

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Objectives: Methotrexate (MTX) is the first line treatment drug for rheumatoid arthritis (RA), however 30% of patients experience MTX inefficacy. With an aim to select patients with inefficient MTX response, we constructed clinical pharmacogenetic index to predict MTX non-response. To obtain the generalization of the developed index in other populations we validated it on a group of Serbian RA patients.

Methods: In total 110 Slovenian patients were analysed by LASSO penalized regression to select variables associated with DAS28 after six months of MTX monotherapy among clinical factors and 34 polymorphisms coding for MTX transporters and metabolizing enzymes. Clinical pharmacogenetic index was constructed from penalized regression coefficients with absolute value above 0.05, cross-validated and independently validated on Serbian group, consisted of 133 patients.

Results: Clinical pharmacogenetic index for prediction of DAS28 after six months of MTX monotherapy in Slovenian RA patients consisted of DAS28 at diagnosis, presence of erosions, MTX dose, SLC19A1, SLCO1B1, TYMS and AMPD1. It correctly classified 69% of Slovenian patients and explained 30% of variability in DAS28 after six months of MTX monotherapy. By cross-validation explained variability remained 29%. It correctly classified only 22.5% of Serbian patients and thus poorly predicted DAS28 after six months of MTX monotherapy in another population.

Conclusions: We developed clinical pharmacogenetic index of DAS28 after six months of MTX monotherapy in Slovenian RA patients by combining clinical and genetic variables. Index did not perform well on Serbian patients, presumably due to the differences in clinical management between the two groups.

P10

Examination of the influence of the CYP2C19 c.-889T>G gene variant on clopidogrel treatment and CYP2C19 gene expression

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Introduction: The CYP2C19 gene encodes an enzyme responsible for metabolism of vast majority clinically used drugs among which are antiplatelet drugs, antidepressants, proton pump inhibitors, anticonvulsants. Clopidogrel is a prodrug and requires conversion into an active metabolite before it can exert its antiplatelet function [1]. This conversion is done by hepatic CYPs in a two-step oxidation process and most of it is accomplished by CYP2C19 [2]. Pharmacogenetics studies have shown that variants in CYP2C19 promoter region influence gene transcription and as a consequence change pharmacokinetics, drug efficiency and adverse drug effects [3,4]. The aim of this study was to examine the influence of rare and poorly investigated -889T>G variant on CYP2C19 promoter activity and clopidogrel treatment.

Materials and methods: Study population included 121 patients who underwent Percutaneous Coronary Intervention and were given Clopidogrel daily one year after the procedure. All in hospital bleeding events were assessed using standard medical criteria: Bleeding Academic Research Consortium (BARC) and Thrombolysis in Myocardial Infarction (TIMI) bleeding criteria. Genotyping was performed by direct DNA sequencing. In order to analyse influence of the -889T>G variant on the promotor activity, the 1705 bp of the promoter region of the CYP2C19 gene was cloned into pGL4.1 luciferase reporter vector and transfected in HepG2 cell line. To estimate the basal activity of wild type and -889T>G promoters dual luciferase assays were performed. Further, to inspect weather CYP2C19 c.-889T>G variant is in haplotype with any other variant within coding region we sequenced all 9 exons of CYP2C19 in the 16 patients, who were carriers of the targeted variant.

Results: Whereas significant association between -889T>G variant and TIMI bleeding (p= 0.01) was observed, further we focused on possible influence of -889T>G variant on CYP2C19 expression. Luciferase assays showed that basal activity of promoter containing -889T>G variant was about 33% decreased compared to wild type promoter ($p<0.05$). Analysis of coding region shows that in Serbian population CYP2C19 c.-889T>G variant is not in haplotype with CYP2C19 c.636G>A as it is indicated in The Human Cytochrome P450 (CYP) Allele Nomenclature Database (http://www.cypalleles.ki.se/cyp2c19.htm).

Conclusion: Since, association between CYP2C19 c.-889T>G variant and TIMI bleeding indicates that this variant may elevate gene expression and in vitro results of promoter activity have shown that same variant causes decreased promoter activity, we need further analysis to explain this discrepancy. However, this disrepancy is not surprising considering the fact that the interindividual variability in the response to clopidogrel is multi-factorial and largely influenced by environmental, clinical and other genetic factors. Beside different variants within the coding region as well as within introns could contribute to the CYP2C19 gene expression and activity. Therefore, the next step should be reconstruction of the CYP2C19 haplotypes in Serbian population and their influence on gene activity.

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P11

Renal cell carcinoma: Clinicopathologic characteristics and evaluation of prognosis

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Renal cell carcinoma (RCC) is the predominant type of kidney cancer in adults, which accounts for 90% of all cases. Higher incidence and mortality rates prevail in Europe, North America and Australia, while are significantly lower in Asia and Africa. According to histologic subtype, the RCC could be classified as conventional (clear cell), papillary, chromophobe, Xp11 translocation carcinomas and unclassified subtypes. The majority of cases are of conventional subtype (75%), while papillary and chromophobe subtypes account for 15% and 5% of all cases, respectively. Previous studies recognized TNM stage and Fuhrman grade as prognostic factors. Our goal was to analyze the association between histologic subtypes and clinicopathologic characteristics in patients with RCC and to evaluate their survival rate.

The study included 180 patients with diagnosed RCC who underwent radical nephrectomy. Fuhrman grading system and Heidelberg classification were used for grade and hystologic features assessment by pathologists at the Medical Faculty, University of Belgrade. Informed consent was obtained from every patient. The collected data included age, gender, grade, tumor size, hystologic subtype and survival. Contingency tables and t-test were used to compare clinicopathological and quantitative variables, respectively. Survival was determined from the date of surgery until death or until last follow-up appointment. Survival distributions were performed by Kaplan-Meier method and groups were compared using log-rank statistics. The $p<0.05$ was considered significant.

One hundred forty one (78.3%), 28 (15.6%) and 11 (6.11%) were classified as conventional clear cell, papillary, and chromophobe carcinoma, respectively. The association was found among histologic subtype, gender and grade. A male predominance was observed in clear cell (66.67%) and papillary (67.86%) tumors, whereas women had higher frequency of chromophobe tumor subtype (72.73%). Fuhrman grades 1 and 2 were found in 66.67%, 42.86%, and 81.82% of the three histological subtypes, respectively ($p=0.0251$).

Sixty five patients with clear cell (n=48; 73.85%) or papillary carcinomas (n=17; 26.15%) were available for survival analysis. No survival difference was observed between these two groups (log -rank $p=0.45$), and the five-year survival rates were 74.96% and 62.57%, respectively. When histologic subtypes were adjusted to gender no significant survival difference was found between both tumor types (log-rank $p=0.91$ and 0.17 for men and women, respectively). Similarly, when stratifying histology according to tumor grade, the two histologic groups had the same outcome for grade 1 and 2 tumors (log-rank $p=0.27$), and also for grade 3 and 4 tumors (log-rank $p=0.40$).

The study showed that although histologic subtypes were correlated to gender and grade and were significant as single variables, both clear cell and papillary tumors were not found to have a significantly different outcome. Since each type of tumor has its own genetic and protein features, the understanding of the molecular basis of histologic subtypes will provide the greater insight into the various tumorigenic pathways of renal carcinogenesis.

P12

Genotyping of hypertrophic cardiomyopathy patients in Serbia by next generation sequencing

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Introduction: Hypertrophic cardiomyopathy (HCM) is autosomal dominant disease of the myocardium, which is characterized by myocardial hypertrophy. HCM is very common with incidence 1 per 500 and can affect people of any age. Its predisposition to sudden cardiac death provides a need to identify disease-specific gene variants in affected individuals, following testing of at-risk family members. Almost all mutations detected in HCM patients are 'private mutations', identified in one or only a few families [1].

Material and Methods: In this study were enrolled 47 patients with hypertrophic cardiomyopathy from Serbian population. All patients had left ventricular hypertrophy in the absence of an underlying systematic condition or other cardiac disease. We analyzed 46 genes involved in inherited cardiomyopathy by targeted next generation sequencing (NGS). Paired-end sequencing was performed on MiSeq v2. Illumina VariantStudio 2.2 software was used for data analysis. Annotation and filtering of variants were done based on their quality, frequency (✣ 1%) and consequence (non-synonymous, slice region or null variant).

Results: In 42 patients we identify 76 variants. More than 1 variants was detected in 21 patients. Pathogenicity of detected variants was assessed according to guidelines of American college of medical genetics and genomics. Using these rules we identify 20 pathogenic or likely pathogenic variants. 16 of them lie within sarcomeric genes and 4 in other genes implicated in inherited cardiac disorders.

Conclusions: Based on these results we can conclude that addition of nonsarcomeric genes in our testing of HCM patients improve diagnostic yield from 35% to 43%, however it's drawback is increase in number of VUS variants from 34% to 49%. This study represents the first multi gene analyses of HCM patients with Serbian origin. The utilized cardiomyopathy panel for targeted resequencing of HCM patients enables high-throughput and rapid turn-around-time from sample to clinical interpretation.

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P13

Genetic determinants of Parkinson's disease: can they help to stratify the patients based on the underlying molecular defect?

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Introduction: Parkinson's disease (PD) is a complex progressive neurodegenerative disorder with a relatively strong genetic background. Within the preparative phase of the ARTEMIDA project, we reviewed the current knowledge on the genetics of PD in particular regarding the genetic factors that could be indicative of the underlying molecular defect.

Results: In our review we focused mainly on the more frequent idiopathic form of PD, which has a rather polygenic origin in comparison to monogenic familial PD, which accounts for approximatelly 5-10 % of all PD cases. According to some studies one third of PD risk is genetically heritable, however only 10 % of associated genes have been discovered so far [1]. Information on novel risk genes is coming from several genome-wide association studies (GWAS) and their meta-analyses. The pathology of sporadic PD is somehow overlapping with the monogenic familial forms, as SNCA and LRRK2 were simultaneously found to be both causal genes and risk factors [2]. GWAS that have been performed so far enabled geneticists to establish 28 single nucleotide polymorphisms (SNPs) as PD risk factors within 24 loci [3]. The identified loci take part in numerous cellular pathways that may contribute to PD pathology: protein aggregation, protein and membrane trafficking, lysosomal autophagy, immune response, synaptic function, endocytosis, inflammation and metabolic pathways are among the most important ones [4]. The identified SNPs are usually located in the non-coding regions and their functionality remains to be determined, although they presumably influence gene expression. It is important to be aware of a very low contribution of a single genetic risk factor to PD development, hence the idea of a cumulative nature of genetic risk factors [5].

Conclusions: Better understanding of PD will help to elucidate the underlying pathological processes. Such knowledge would help physicians to recognize the disease in its earliest stage and open the opportunity for starting the medication sooner to slow down its progress or maybe even prevent it. Moreover, the idea is to stratify the PD patients according to their genetic fingerprint to properly personalize their treatment and to undertake particular supportive measures.

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P14

Pharmacogenetics of Parkinson's disease Planning of a clinical-pharmacogenetic model for personalized treatment of Parkinson's disease

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Background: Parkinson's disease (PD) is the second most common neurodegenerative progressive brain disease with increasing prevalence in aging population. The etiopathogenesis of the disease is not fully elucidated yet, but it is known that many cellular procesess are involved in the pathology, such as inflammation, antioxidant defense, neuronal development, lipid metabolism, signalling pathways, calcium homeostasis, protein degradation, mitochondrial and lysosomal function and synaptic transmission. It is treated symptomatically with dopaminergic therapy - levodopa and dopamine agonists. Occasionally, MAO-B inhibitors, COMT inhibitors, amantadine or anticholinergics are used as initial monotherapy or as adjuvant therapy. Many adverse drug reactions (ADRs) can occur during the treatment process, which we cannot predict and/or prevent. Non-motor ADRs, such as nausea, somnolence, hallucinations and hypotension are frequent in dopamine agonist therapy. In comparison, dyskinesias along with motor fluctuations are the most common late ADRs in levodopa treatment. Many pharmacogenetic studies have already investigated PD susceptibility and treatment response, but very few of them combined environmental factors, demographic data and genetic polymorphisms in the algorithm for personalized approach to PD management.

Methods: We are planning a clinical study to assess the combined impact of selected polymorphisms, demographic data and numerous environmental factors on the progression of the disease, ADRs and treatment response. We will perform a retrospective study with longitudinal follow-up. We will collect peripheral blood samples of PD patients and single nucleotide polymorphisms (SNPs) in the genes involved in dopamine synthesis, degradation and transport of dopamine and drug molecules, signalling pathways, release and uptake of neurotransmitters and in the dopamine receptor genes will be genotyped. In the prospective part of the study we will also isolate the exosomes and check their miRNA content at the time of diagnosis and after the treatment initiation. The combined effects of clinical and genetic data will be analyzed using lasso penalized regression analysis.

Implications and conclusions: We hope to identify biomarkers that may predict the efficacy of anti-parkinsonian drugs as well as the risk factors for the occurence and time to occurence of ADRs. If such prediction models would be established they would support personalized treatment of PD.

P15

Characterization of inflammatory and apoptotic molecular markers of active Crohn's disease

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Introduction: Intestinal mucosal damage in Crohn's disease (CD) patients occurs as a result of the deregulation of inflammatory and apoptotic processes. Transcriptional factor NF-ĐB has a key role in transcription of genes that mediate these events. The aim of this study was to elucidate molecular patterns that underlie these processes.

Results: We analyzed expression levels of proinflammatory IL-6 and TNF-Đ, and apoptotic Bcl-2, Bax, Fas and FasL genes in intestinal mucosa and peripheral blood mononuclear cells (PBMC) of 24 patients with active CD and 21 controls, using qRT-PCR methodology. Among these participants, we selected 10 CD patients and 5 controls in order to conduct EMSA analysis of the DNA binding activity of NF-ĐB from the nuclear extracts of donors' intestinal mucosal samples.
Results showed that expression levels of IL-6 and TNF-Đ were significantly increased, while expression level of Bcl-2 was significantly decreased in ileal inflamed mucosa of CD patients. Our results also revealed that the expression level of FasL in PBMC of CD patients was significantly decreased, but only in male patients.
The analysis of the DNA binding activity of NF-ĐB revealed a linkage between decreased level of NF-ĐB binding activity, along with increased expression level of TNF-Đ, and intestinal mucosal fragility. In contrast, increased level of NF-ĐB binding activity and decreased expression level of TNF-Đ were related with intestinal strictures.

Conclusions: Our results demonstrated that the expression profiles of selected proinflammatory and apoptotic genes, as well as the DNA binding activity status of NF-ĐB could be considered as molecular markers of active CD.

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P16

Detection of potential factors for monitoring disease in patients with systemic sclerosis in population of Serbia

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Systemic sclerosis (SSc) is an autoimmune connective tissue disease with unknown etiology. Growing evidence suggests that T-cell proliferation and cytokine secretion play a major role in the pathogenesis of SSc. IL-23, heterodimeric cytokine, is responsible for the differentiation and expansion of Th17 cells from naive CD4+T cells which are characterized by the predominant production of interleukin (IL)-17A/F, IL-21 and IL-22. IL-17 (A:A homo-/A:F heterodimer) can stimulate expression of adhesion molecules on endothelial cells resulting in recruitment, activation and migration of neutrophils and macrophages contributing to autoimmune inflammation in SSc.

The expression level of IL-17 (isoforms A and F) and IL-23 genes was detected in the PBMCs of 63 SSc patients and 13 healthy controls using qRT-PCR. Serum levels of IL-17 and IL-23 were examined using ELISA assay on subset of 31 SSc female patients and 9 healthy female subjects. Our data showed a significant up-regulation of IL-17F mRNA (p=0.006) in patient group. However, there were no statistically significant differences in expression levels of IL-17A and IL-23 gens or serum levels of IL-17 and IL-23 between analyzed groups.

Our study showed possible involvement of IL-17F gene isoform in pathogenesis of SS. The role of IL-23/IL-17 immune axis have not been fully understood in SSc, so larger prospective and longitudinal studies will provide more meaningful information and hints for innovating targeted therapies for patients with SSc.

P17

SLC6A4 5HTTLPR polymorphism affects insulin secretion in patients with polycystic ovary syndrome

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Background: Studies in animal models and cell cultures suggest that the association between the serotoninergic system and basal and glucose-stimulated insulin secretion may be plausible. Our aim was to investigate the association between genetic polymorphisms in serotoninergic system and basal and glucose-stimulated insulin secretion in women with polycystic ovary syndrome (PCOS).

Methods: The study included included 65 female patients with PCOS (aged 30.0 (25.8-36.0), BMI 38.2 (33.5-41.6) kg/m2) and a control group of 94 young healthy female blood donors. Oral glucose tolerance test (OGTT) was performed in PCOS patients and basal and glucose-stimulated blood glucose and insulin levels were measured. Patients and controls were genotyped for 5HTR1A rs6295, 5HTR1B rs13212041 and SLC6A4 5HTTLPR polymorphisms. The statistical analysis was performed using Mann-Whitney test.

Results: Genotype distributions were in accordance with HWE, except for 5HTR1A rs6295 in healthy controls and 5HTR1B rs13212041 in PCOS patients. Carriers of at least one polymorphic 5HTR1A rs6295 G allele had almost three times lower risk for PCOS than homozygotes for the normal C allele (OR = 0.34; 95 % CI = 0.16-0.75; p = 0.008). Genotype distribution of other polymorphisms did not differ significantly between the patients and controls. SLC6A4 5HTTLPR polymorphism was significantly associated with insulin secretion (p=0.030) and with the area under the curve of insulin blood levels during OGTT (p=0.021). None of the investigated polymorphisms was significantly associated with basal or glucose-stimulated blood glucose levels at any point in time during OGTT, or with the basal insulin concentration.

Conclusions: Serotoninergic system may play a role in glucose stimulated insulin secretion in patients with PCOS.

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