Czech Centre for Phenogenomics
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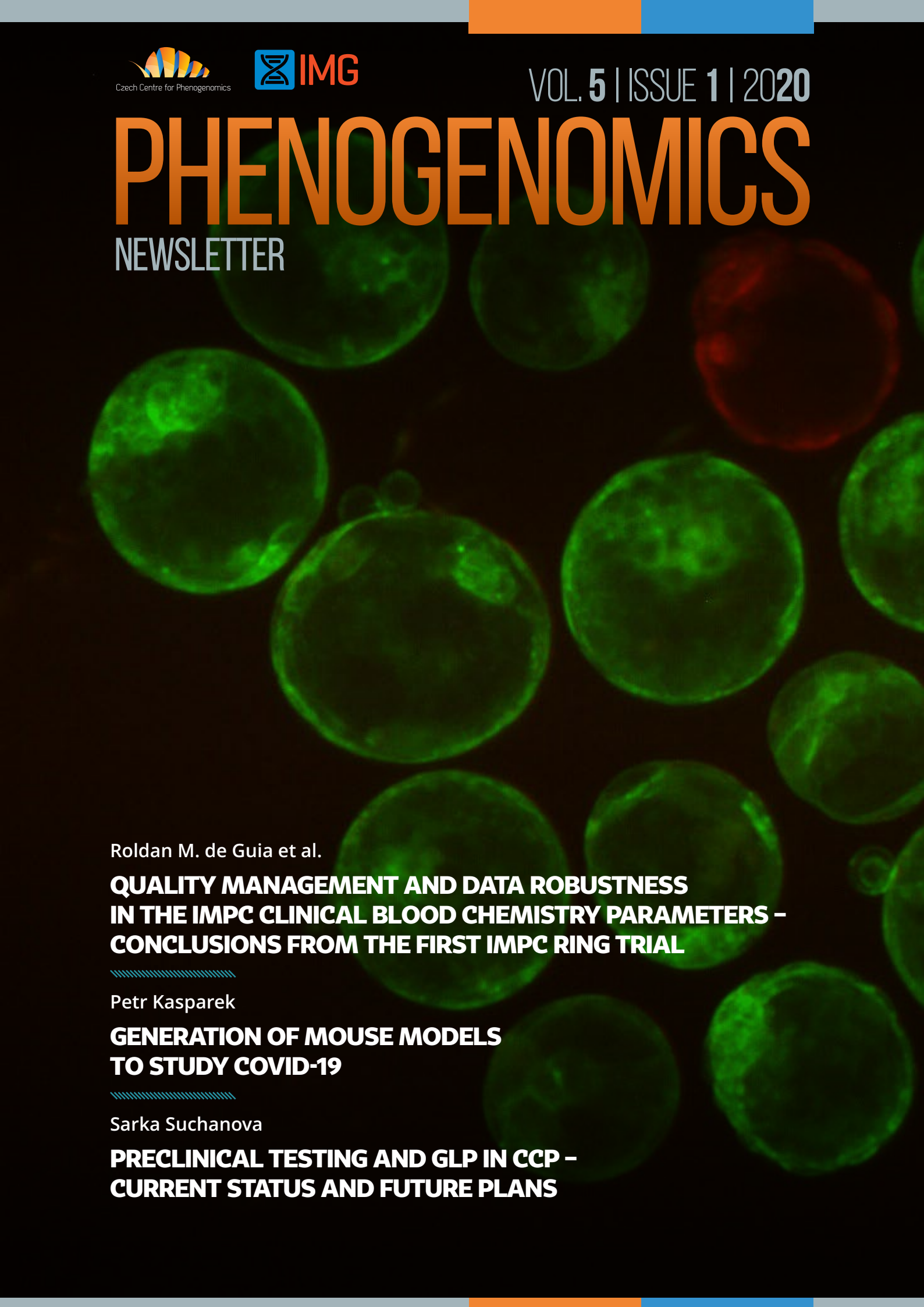
PHENOGENOMICS
NEWSLETTER



Roldan M. de Guia et al.

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Cover Image:
Representative fluorescent image of blastocysts, in which floxed tdTomato allele was converted to GFP upon electroporation with Cre protein with 95% efficiency. (Jenickova et al.)
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The editorial team would like to thank the authors in this issue for their contribution.

INTRODUCTION

Dear reader,

This is the 1st issue of Volume 5 (2020) of the PHENOGENOMICS NEWSLETTER published by the Czech Centre for Phenogenomics (CCP). The PHENOGENOMICS NEWSLETTER features overviews of the services available to researchers and current or potential collaboration partners. Its objective is to highlight recent achievements, introduce new technologies and directions for future development, and allows insights into work and life at the CCP.

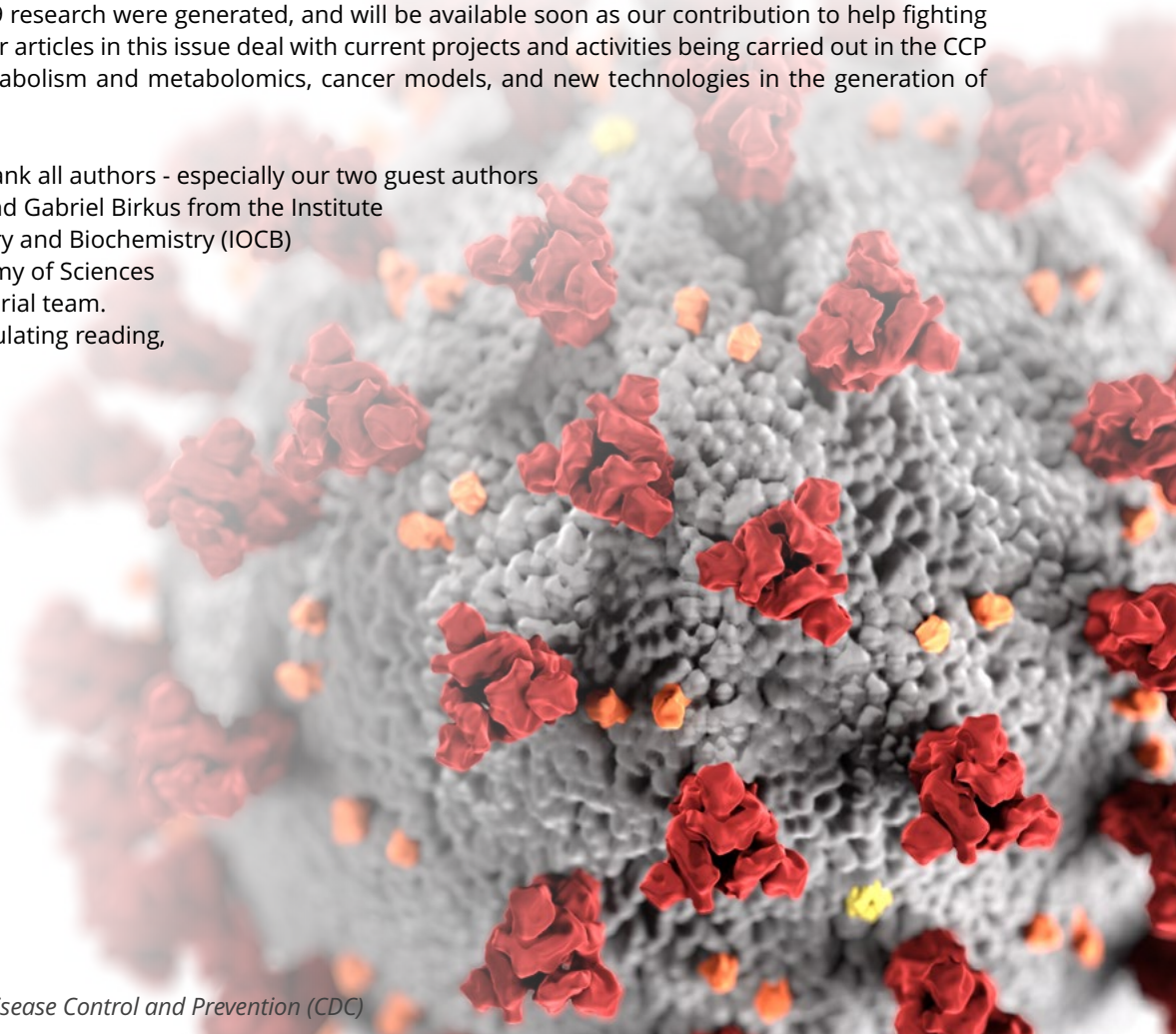
What an exceptional year 2020 has been, with the new SARS-CoV-2 virus spreading across the globe within a matter of weeks, forcing a standstill in many countries so far unknown to this generation. In this situation, biomedical sciences are facing challenges that have rarely been experienced before on this scale. SARS-CoV-2 and the resulting disease Covid-19 are impacting our everyday life. Fortunately, the CCP has endured the pandemic crisis well so far. Fulfilling the aims of large research infrastructures, the CCP managed to continue providing users and collaboration partners with state-of-the-art support for their research. The CCP Training and Education Program was also continued as long as possible by switching to virtual events, especially during the second half of the year. Highlights of this year were an onsite course "Publishing in peer-reviewed journals" and a training workshop on telemetry co-hosted by the CCP. For more information about CCP services and activities, please, visit the CCP new website (www.phenogenomics.cz).

Despite numerous restrictions due to the pandemic, even the 2nd CCP Conference could be organized in September with many outstanding speakers joining from abroad via webcam and headset. Day 1 of this conference had a special focus on preclinical testing. This is to underline that the CCP intends to become more involved in this area soon.

In 2020, the CCP offered several open calls to support researchers in emergencies caused by the Covid-19 pandemic such as the rescue of mutant lines via sperm freezing, etc. However, as you can read in the article by Petr Kasparek, we have also positioned ourselves in the field of Covid-19 research. New experimental mouse models for Covid-19 research were generated, and will be available soon as our contribution to help fighting the pandemic. Other articles in this issue deal with current projects and activities being carried out in the CCP in the areas of metabolism and metabolomics, cancer models, and new technologies in the generation of mouse models.

We would like to thank all authors - especially our two guest authors
Andrea Brazdova and Gabriel Birkus from the Institute
of Organic Chemistry and Biochemistry (IOCB)
of the Czech Academy of Sciences
and the whole editorial team.
We wish you a stimulating reading,

Jan Rozman
editor



Source: Centers for Disease Control and Prevention (CDC)

RADISLAV SEDLACEK ELECTED NEW CHAIR OF THE ESFRI STRATEGIC WORKING GROUP ON HEALTH AND FOOD

Marketa Morska

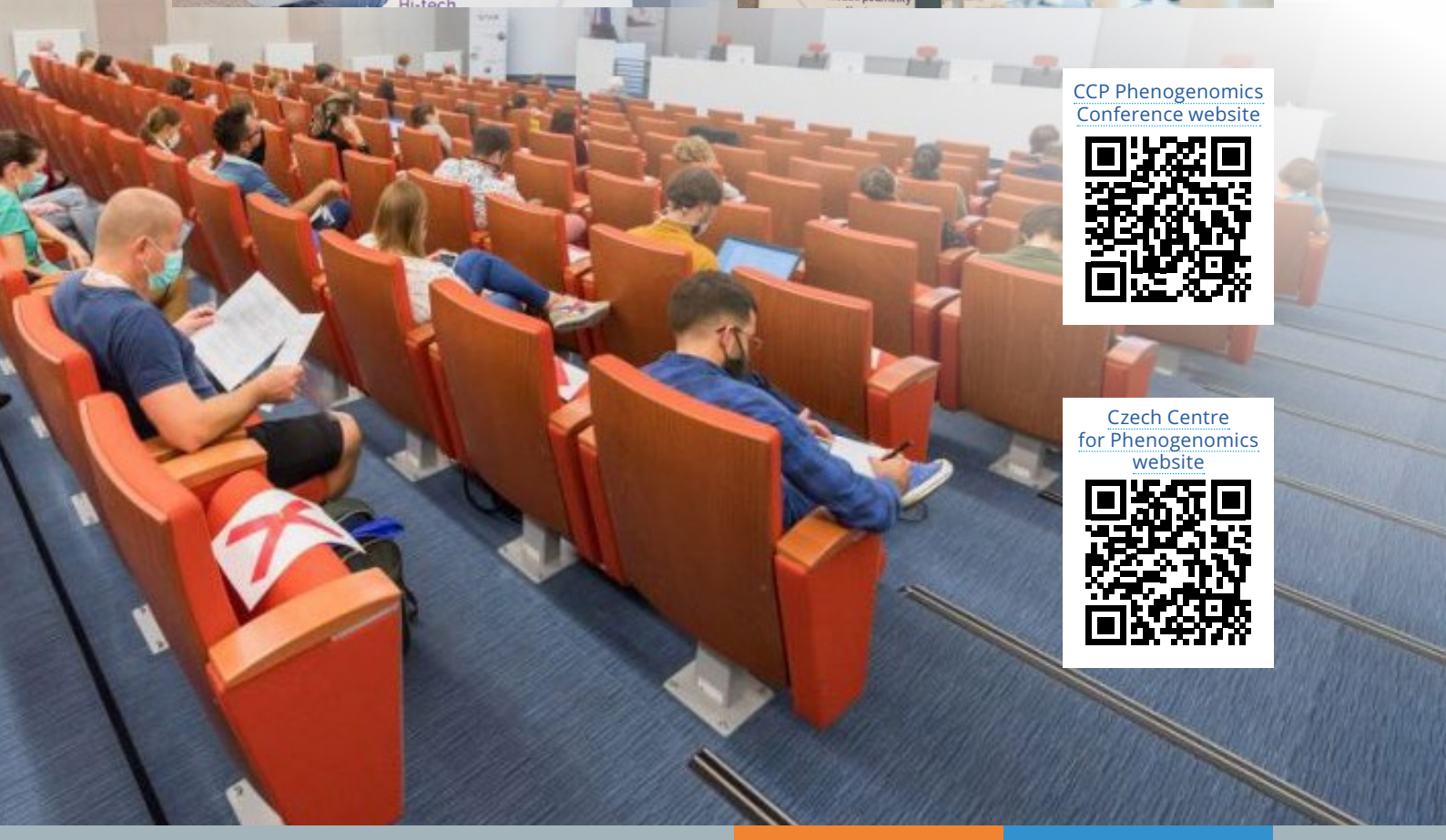
Radislav Sedlacek, the director of the Czech Centre for Phenogenomics was elected as the new Chair of the ESFRI Strategy Working Group on Health and Food in May 2020. The European Strategy Forum on Research Infrastructures (ESFRI) advises EU policy-makers on research infrastructures in Europe and facilitates international actions in that area. ESFRI also issues the periodic Roadmap of pan-European Research Infrastructures, which provides strategic guide for the Member States for future investments. The INFRAFRONTIER (European research infrastructure for the generation, phenotyping, archiving, and distribution of mouse disease models), in which CCP is actively involved, is marked as the ESFRI Landmark, i.e. an advanced research infrastructure creating foundations of competitiveness of the European Research Area. The strategic working group represents the ESFRI expert platform, which focuses on road mapping and landscape analysis of European research infrastructures in the fields of biological and medical sciences and food. The working group also aims to foster mutual collaboration between European biological, medical, and food research infrastructures and other related European initiatives and programs.

More info: www.esfri.eu

2nd CCP PHENOGENOMICS CONFERENCE 2020

Jan Rozman

Preclinical research, immunology, and the motto "From CCP Users For CCP Users" were the main topics of this year's 2nd CCP Phenogenomics Conference on September 17th and 18th. Despite necessary protective measures due to the Covid-19 pandemic, an outstanding event could be organized highlighting the scientific impact of the Czech Centre for Phenogenomics as a large European Research Infrastructure. The conference took place at the BIOCEV campus in Vestec as an on-site event with the participation of high-profile remote international speakers. Altogether, the conference was attended by 90 on-site participants, 17 remote speakers, and online streaming was watched by 30 participants on average. On the first day the theme "From Chemistry via Preclinical Pipeline to Therapeutics" emphasized the translation of basic research into clinical application. A wide range of speakers from medicinal chemistry, environmental toxicology, bioinformatics, animal model development, and preclinical testing gathered either in-person or virtually. As only one example for the excellent talks, Eckhard Wolf from Technical University Munich introduced his work using genetically engineered pigs as alternative models for diabetes research. During this day, it became clear that there are numerous connecting points between these areas and that a new network of the involved research groups could help to boost clustering in the area of preclinical evaluation in the Czech Republic and several other European countries. The second day had a special focus on CCP's users and collaboration partners working in the field of immunology, hematology, the genetic base of disease, and neurobiology. Speakers presented exciting research results that were obtained with support from the CCP. Similar to the last year, both the industry and the poster exhibition stimulated numerous interesting contacts and conversations between participants.



CCP Phenogenomics Conference website

Czech Centre for Phenogenomics website

GENERATION OF MOUSE MODELS TO STUDY COVID-19

Petr Kasparek

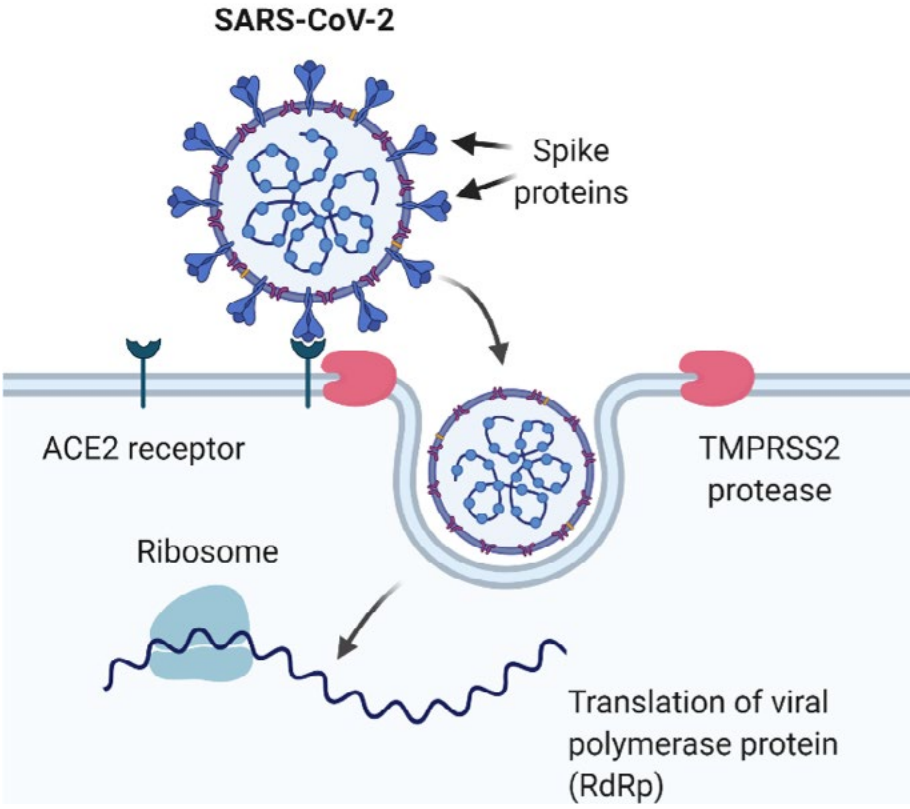
Coronavirus disease 2019 (COVID-19) has recently spread around the globe and has become one of the most severe threats to public health. Instantly, COVID-19 with its causative agent SARS-Cov-2 came into the scientific spotlight and several research laboratories worldwide joined their efforts to understand its etiology, underlying molecular mechanisms and to develop potential therapeutic approaches. Animal models offer several advantages to study COVID-19, allowing for a complex in vivo investigation of the disease andpreclinical testing of drugs and vaccines. Yet, only a limited number of suitable mouse models was available for a wide research community by the onset of the pandemic. Czech Centre of Phenogenomics (CCP), being one of Europe's largest facilities in mutant mouse production, has swiftly taken steps to develop suitable and broadly available mouse mutants to study COVID-19. Here, we provide an overview of the COVID-19 related models currently under development, and models that have been already produced at CCP.

TARGETING ACE2

Angiotensin converting enzyme II (ACE2) is a transmembrane metalloproteinase, previously established as an important player in the regulation of blood pressure. ACE2 is expressed in lungs, heart, liver, kidney and intestine. By 2003, it had been identified as the main entry point into cells for severe acute respiratory syndrome coronavirus (SARS-CoV). Specifically, the spike proteins of SARS-CoV bind membrane-associated ACE2, which is followed by translocation of both, the virus and the enzyme into the cell. Soon after the COVID-19 outbreak in late 2019, it was shown that ACE2 plays a very similar role in the SARS-Cov-2 infection process.

HUMANIZED HACE2 MODELS

Human and mouse ACE2 enzymes share about 80% homology and a similar expression pattern. However, the mouse Ace2 variant has significant amino acid variations in the viral receptor binding domain, therefore SARS-CoV and SARS-CoV2 infections are mediated with dramatically lower efficacy. Consequently, wild type mice are highly resistant to these viruses and their use in SARS or COVID-19 research is limited. An obvious strategy to circumvent this problem was generation of mouse models carrying human Ace2 variant in their genome. Tseng et al. developed the first such model in 2007, carrying the human Ace2 gene expressed from a strong and ubiquitous CAG promoter [1]. Indeed, mice expressing human Ace2 gene were highly susceptible to SARS infection, dying within 4-8 days upon infection (in contrast to surviving wild-type animals). However, the primary cause of death was severe inflammatory response in the brain – a symptom not observed in human patients. Poor recapitulation of the human disease was likely due to strong and unspecific transgene expression. This limitation was partially overcome in a keratin18 promoter-based hAce2 mouse model, which directed the human receptor strictly to epithelia, including epithelial cells of the respiratory tract [2]. Challenged by SARS-CoV, K18-hAce2 mice developed a severe infection beginning in airway epithelium that, similarly to the previous hAce2 model, spread to the brain and resulted in a lethal phenotype. Arguably the most accurate model was based on hAce2 expression driven by the mAce2 promoter fragment. Infected mice carrying mAce2 promoter-hAce2 transgenic construct showed severe interstitial pneumonia with extrapulmonary organ damage, closely resembling the human disease [3].



Schematic representation of the SARS-CoV-2 infection.

Although none of these models reproduced the full disease spectrum observed in SARS-CoV infected patients, they were crucial to identify the important role of the Ace2 receptor in SARS disease. However, mouse models carrying a human Ace2 variant were not broadly available by 2020, as SARS did not present an immediate threat anymore and the general interest in studying this virus had faded. This has changed dramatically after the COVID-19 outbreak due to apparent similarities between SARS-CoV and SARS-CoV-2 viruses. Indeed, initial experiments using hAce2 mice confirmed that the human receptor is an efficient gateway into the cells, which is also truefor the SARS-CoV-2 virus. K18-hAce2 mice infected by the "novel coronavirus" showed efficient viral replication in the heart, brain, kidney and spleen, which was in contrast to wild type mice [4].

All the previously described hAce2 models, based on random integration of the transgenic cassette, showed replication of the virus in cells that are not affected in human patients. This is possibly due to nonphysiological expression of the transgene driven by artificial promoters. The CRISPR revolution in genome editing that occurred over last decade has allowed the generation of more complex models than ever before. Targeted insertion of a transgene, such as hAce2, in any mouse genomic locus presents a feasible challenge that can be achieved in a relatively short time-frame and with reasonable costs. Considering hAce2 driven by mAce2 promoter fragment rendered a suitable mouse model for SARS, we believe that insertion of hAce2 into endogenous mAce2 locus may provide even more precise recapitulation of the desired expression pattern, preserving all the important regulatory elements required for mAce2 expression. Furthermore, the replacement of the endogenous gene by the human variant would prevent co-expression of both possibly interfering proteins. Validity of this approach was recently shown by Sun et al. that employed a very similar strategy to generate a hAce2 model suitable for studying SARS-CoV-2 infection [5]. **CCP researchers initiated the generation of such a model by April 2020, and we expect to obtain the first experimental animals by fall 2020. Additionally we are developing a conditional model allowing hAce2 expression triggered by a tissue–specific CRE driver to study the impact of the infection in specific organs and cell types.**

MODELING ACE2 POLYMORPHISM IN MICE

It has been proposed that ACE2 gene polymorphism, ACE2 mRNA expression and human ACE2 protein polymorphism influence SARS-CoV-2 susceptibility and therefore the outcome of COVID-19 disease. Several isoforms of Ace2 have been identified in different populations, including SNP variants in the regions critical for interactions with the viral Spike protein [6]. Humanized mouse lines carrying different population variants of the Ace2 gene could be used to experimentally verify the relationship between Ace2 polymorphism in humans and disease severity. Our, **CCP team has generated targeting tools to create human Ace2 variant knock-in mouse lines. These mice can be created once the general targeting strategy for Ace2 humanization is experimentally verified in late 2020.**

ACE2 KNOCK-OUT MODEL

A number of promising COVID-19 therapies are focused on targeting the Ace2 receptor. Thus, it is desirable to evaluate A) the potential of Ace2 blocking to prevent viral entry into the cells and B) unwanted off-target effects caused by Ace2 deficiency. Ace2 deficient mutant mice provide an excellent tool for these types of experiments. **Ace2 knock-out mice were generated at CCP in June 2020 and are currently available for a broad scientific community.**

TARGETING TMPRSS2

It has been recently shown that the entry of SARS-CoV-2 requires not only binding to Ace2 receptor, but also priming of its Spike protein by a transmembrane protease TMPRSS2 [7]. TMPRSS2 ablation was previously shown to partially rescue a severe lethal phenotype upon SARS-CoV infection as it prevents efficient viral replication and dissemination, and leads to less severe immunopathology when compared to wild type mice. TMPRSS2 KO mice can be used in the research of COVID-19 infections to investigate the pathophysiological role of TMPRSS2 and to validate TMPRSS2 as a potential therapeutic target. **TMPRSS2 knock-out mice were generated by our CCP team in June 2020 and are currently available for a broad scientific community.**

In conclusion, CCP has already generated two mutant models tailor-made for COVID-19 research and additional models are currently in production. Furthermore, CCP researchers are ready to prioritize production of additional COVID-related models upon specific requests.

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CALL TO SUPPORT COVID-19 RESEARCHER PROJECTS BY PRIORITY ACCESS TO CCP

The Czech Centre for Phenogenomics (IMG CAS) offers immediate support to COVID-19 research projects by priority access to the national large research infrastructure. The CCP generates rodent models related to diagnosis, treatment and prevention of COVID-19 virus infection (e.g. several serine protease mouse mutant lines). Project proposals can be submitted by email to ccp@phenogenomics.cz

Proposals will be evaluated and conducted with highest priority.

AVAILABLE RESOURCES, SERVICE & SUPPORT:

1| Generation of rodent disease models (knockout, humanized, etc.), relevant for COVID-19 research. In particular, the CCP can offer Ace2 and Tmprss2 KO mice. Several serine protease mouse mutant lines are also available (Prss35, Prss12, Prss21, Prss33, Prss55, Prss37, Prss47, Prss54, Prss48, Prss44, Prss57, Klk12, Klk15, Klk11, Klk13, Klk10, Klk8, Klk5). A hAce2 mouse model and mutant line with conditional overexpression of hAce2 are currently generated.

2| Standard cryopreservation and archiving services (sperms and embryos) are maintained.

3| Advanced secondary phenotyping pipelines relevant to lung inflammation and fibrosis (including state-of-the-art imaging, immunology, lung function).

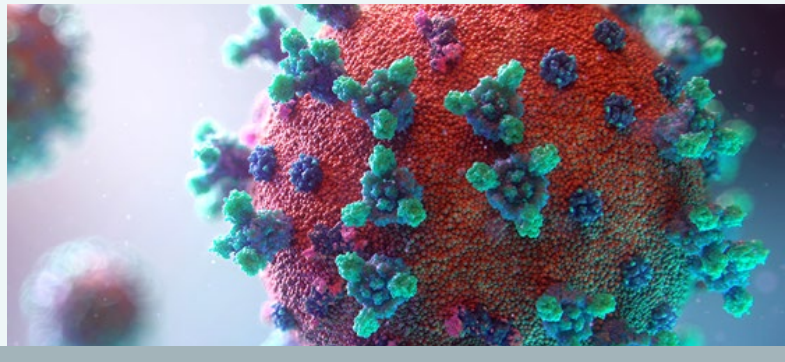
4| Pre-clinical testing (lung inflammation and fibrosis models – clinical biochemistry and histopathology are conducted under GLP conditions).

5| Metabolomics and MALDI imaging from tissue samples and biopsies depending on capacities.

Prioritization call in place to support projects investigating the SARS-CoV-2 infection and its treatment.

Contacts:
General service contact: ccp@phenogenomics.cz

Specialized service contacts:
ccp-tam@img.cas.cz
(model generation, archiving & distribution)
ccp-pm@img.cas.cz
(phenotyping & preclinical service)



COST-EFFECTIVE GENERATION OF MICE BASED ON CRE/LOXP-SYSTEM BY ZYGOTE ELECTROPORATION OF CRE PROTEIN

Silvia Petrezselyova & *Petr Kasparek*

The first genetically modified mouse capable of transferring its modified genome to offspring was developed in the early 1980s (1, 2). Since then, multiple genetic modification techniques have been developed to modify rodent genomes, which led to great savings and the acceleration of research projects. Today's gold standard for creating "knockouts" or "knockins" is to edit the genes by pronuclear microinjections of fertilized oocytes with either DNA, mRNA or ribonucleoprotein complexes. Nevertheless, microinjection techniques take a considerable amount of time to perform and require extensive training for obtaining high rates of transgenesis and embryo survival. The addition of sequence-specific nucleases such as CRISPR/Cas9 to the genome editing toolbox has propelled the search for novel delivery approaches that can bypass the need for microinjection(3). Indeed, several laboratories have recently developed in vitro electroporation-based methods that deliver nucleic acids and Cas9 protein to pre-implantation embryos, thus introducing a new era of generating genome-edited animals in a more simplified manner.

Transgenic Unit of the Czech Centre for Phenogenomics under the direction of Petr Kasparek, Ph.D. has grown in recent years to offer expanded gene and genome engineering services to researchers by applying the latest cutting-edge technologies such as TALENs, CRISPR/Cas9(Cas12a)-based tools, Easi-CRISPR and others. The introduction of specific mutations by electroporation of zygotes is preferentially used over conventional pronuclear injections and other new transgenic technologies are developing to simplify and accelerate the work. The transgenic team in collaboration with the Laboratory of Structural Biology, IBT AS CR has streamlined a method for generation of mouse models based on Cre/loxP conversion, which has been recently accepted in Methods (4). Cre recombinase mediates recombination between two target sites, named loxP, through the spacer region (5). The described protocol is applicable to generate knockouts or reporter mice from EUCOMM/KOMP lines (6, 7) (Fig. 1A) and theoretically from any floxed (flanked by loxP) mice. The simplified strategy primarily relies on direct delivery of Cre protein into mouse zygotes via electroporation with nearly 100 percent efficiency (Fig. 1B) and brings several advantages. It simplifies the work, lowers the cost of generating knockout mice and saves time. While a conventional workflow for floxed allele conversion requires a two-step breeding strategy and takes up to six months (7), the new approach shortens the whole process to one month. Furthermore, Cre protein delivery by electroporation was also shown to be suitable for allelic conversion in primary cells derived from conditional mouse models.

The electroporation-based method allows the delivery of practically any recombinant protein to the cells. Implementing other site-specific recombinases like Flp (recognizing two FRT sites; Fig. 1A) and Dre (recognizing rox sites; (8)) will enable the development of more sophisticated mouse models in a relatively short time. Once these recombinases will become available, our facility will be provided with new options for genome engineering and strategies.

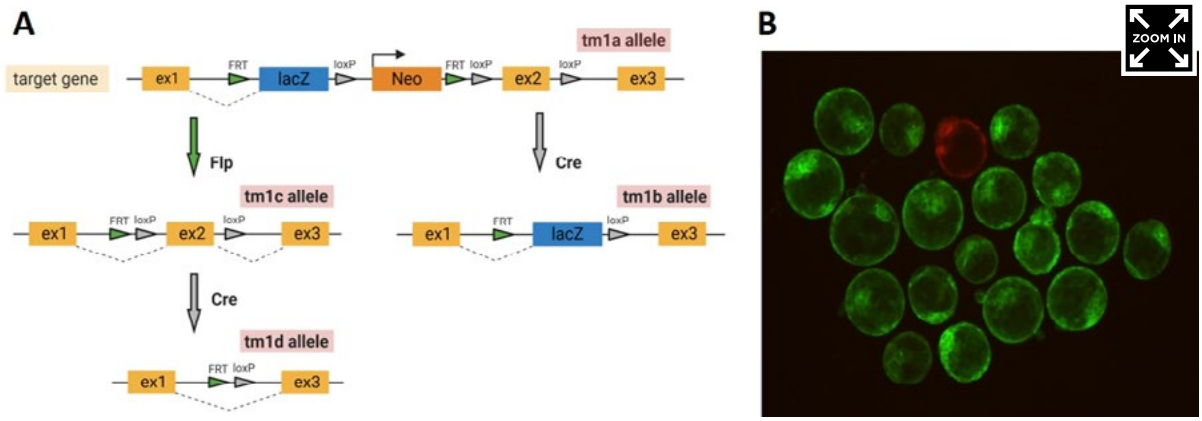


Figure 1: (A) EUCOMM/KOMP gene targeting strategy. Schematic adapted from (6). (B) Representative fluorescent image of blastocysts, in which floxed tdTomato allele was converted to GFP upon electroporation with Cre protein with 95% efficiency.

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ANTI-CANCER THERAPY BASED ON A NOVEL CYCLIC DINUCLEOTIDE AND ITS PRODRUG

Andrea Brazdova & *Gabriel Birkus*

Cytosolic dsDNA sensing is one of the vital innate immunity mechanisms that protect organisms from cell damage and pathogens. Multiple proteins detect the presence of dsDNA in the cytosol including IFI16, AIM2, DDX41, LRRFP1 and cGAS, and trigger different signaling cascades.[1] Among them cGAS (cyclic-GMP-AMP Synthase) which, after binding to dsDNA, catalyzes a reaction of GTP and ATP and produces a cyclic dinucleotide (CDN) called cyclic-GMP-AMP (2'3'cGAMP).[1] This second messenger then binds to a homo-dimeric adaptor protein called Stimulator of Interferon Genes (STING). STING undergoes a conformational change, which allows activation of TANK-binding Kinase 1 (TBK1) and Inhibitors of kappa B kinases (IKKs).[1] TBK-1 and IKKs further activate transcription factors IRF3 and NFκB, which turn on expression of type I interferons and pro-inflammatory cytokines such as TNF-α and IL-6. The released cytokines then trigger immune defense against invading pathogens via their interaction with both immune and non-immune cells. Furthermore, activation of the cGAS-STING pathway by host dsDNA was shown to play an important role in the induction of anti-tumor adaptive immunity. Spontaneous CD8+ T cell priming against tumor specific antigens is defective in STING knock-out mice, and activity of anti-PD1 mAbs is dramatically impaired in $STING^{KO}$ mouse tumor models, making the pathway a prime target for anticancer and antiviral therapy[2].

Unfortunately, 2'3'-cGAMP is labile toward hydrolysis by phosphodiesterases present in biological fluids and the two negative charges on the phosphonic oxygen atoms limit its diffusion across cellular membranes. At the Institute of Organic Chemistry and Biochemistry we assembled a cross functional team of medicinal chemists, biologists, crystallographers and computational chemists and embarked on a rational design of novel CDN analogues that would address these limitations. First, we replaced phosphodiester bonds in the natural CDN with carbon-phosphate (so-called phosphonate) bonds that are resistant to hydrolysis by phosphoesterases. We prepared phosphonate CDNs by conventional chemical synthesis, but we also used an enzymatic synthesis by employing mouse cGAS. Luckily, the enzyme is quite promiscuous and can accept many ATP/GTP analogues as substrates.[3] Second, we masked negative charges on phosphates with bio-labile lipophilic prodrug moieties that allow free diffusion of CDNs across cellular membranes (Figure 1). As a consequence of this effort we were able to

prepare compounds that were two – three orders of magnitude more potent in vitro than 2'3'cGAMP. For an in vivo study, we had to pick compounds with good pharmacokinetic properties. Unfortunately, mouse plasma is known to have high levels of carboxylesterases that can readily cleave the carboxylesterase bonds present in prodrugs. Indeed, with exception of a few compounds, the vast majority of them had a half-life of less than two minutes but were stable in human plasma.

The literature shows that intra-tumoral (IT) administration of CDNs induces potent anti-tumor immunity and tumor growth inhibition[4]. As mentioned above, STING agonists act as adjuvants stimulating innate immune system with subsequent induction of adaptive immunity. Thus, it makes sense to inject them to the site with the highest concentration of tumor specific/associated antigens. We therefore performed a pilot study at the Czech Centre for Phenogenomics in Prague with a parent CDN and its optimized prodrug in a 4T1 syngeneic mouse model. Compounds were applied IT three times per week for one week when tumors reached volume of about 100 mm^3. As anticipated, at the equimolar doses the prodrug showed much more robust tumor growth inhibition than the patent CDN (data not shown). Moreover, the prodrug also induced more potently adaptive anti-tumor immunity represented by the presence of tumor-specific CD8 T cells in mouse blood (Figure 2).

Thanks to the professional approach of the Czech Centre for Phenogenomics, particularly of Phenotyping Module – PDX/Cancer model department, we were provided with extensive data on the first set of our drug candidates. When the in vivo study was initiated in late March of this year, we faced the risk of its cancellation due to the COVID-19 pandemic. To finish in vivo experiments, our colleagues from PDX/Cancer model department had to overcome extra obstacles of working under restricted conditions. Despite these complications and thanks to their dedication, we not only established the syngeneic 4T1 mouse cancer model, but also obtained valuable data regarding anti-tumor effectivity of our compounds.

Following the successful pilot study in cooperation with PDX/Cancer model department, we intend to obtain more data to support our preliminary results. We plan to test the activity of our proprietary STING agonists in various syngeneic mouse cancer models and study their activity in combination with immune checkpoint inhibitors.

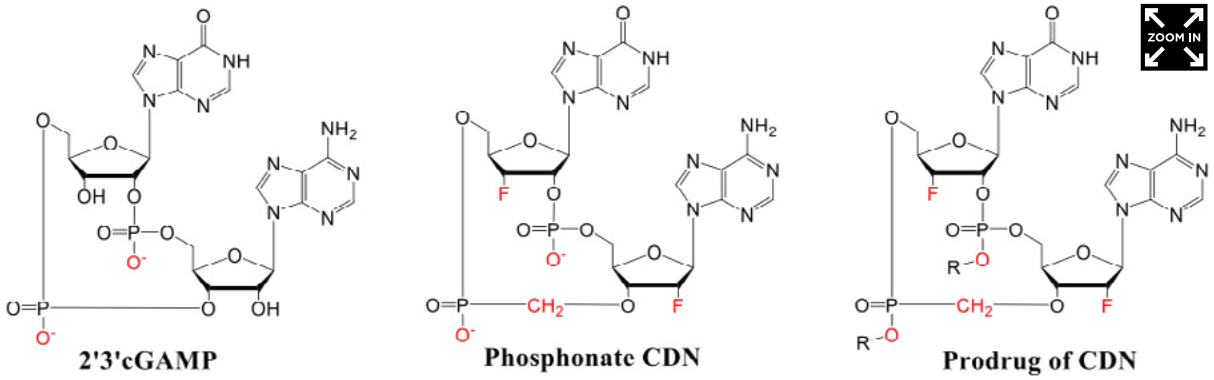

Figure 1: Structure of 2'3'cGAMP, a phosphonate CDN and its prodrug

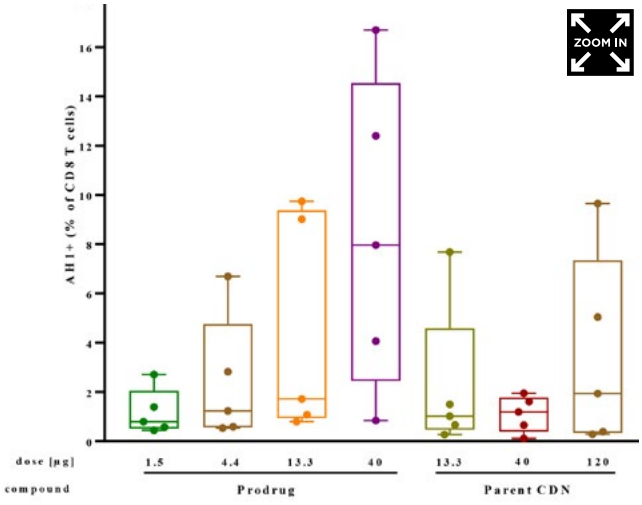

Figure 2: Effect of the parent CDN and its prodrug on tumor specific adaptive immune response. Seven days post last injection, PBMCs from each mouse were assessed for the frequency of CD8+H-2Ld-AH1 Tetramer+ (AH1+) cells by flow cytometry.

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QUALITY MANAGEMENT AND DATA ROBUSTNESS IN THE IMPC CLINICAL BLOOD CHEMISTRY PARAMETERS – CONCLUSIONS FROM THE FIRST IMPC RING TRIAL

Roldan M. de Guia, *Karel Chalupsky* & *Jan Rozman*

The International Mouse Phenotyping Consortium (IMPC) aims to generate and phenotype a murine, knockout mutant model for every protein-coding gene, with the vision to compile a comprehensive catalogue of mammalian gene functions. Currently, phenotyping data for 7022 mutant lines is available that can be interrogated for alterations indicative for human disease. The standardized phenotyping pipelines cover major disease-related areas that match to human clinical investigations related to development, metabolism, cardiovascular system, behavioral and neuromuscular functions, vision, hearing, reproduction, hematology and immunology, and clinical chemistry.

Clinical chemistry has the primary purpose of performing analytical procedures for the quantification of different ions, enzymes, biomolecules, and other serological components of a biological material. The assay results yield accurate and precise information that can aid in diagnosis and treatment of diseases. The IMPC clinical chemistry protocol involves determination of biochemical parameters in plasma obtained from terminal blood. Member centers utilize clinical chemistry analyzers to measure these analytes. The analyzers are similar to those human clinical laboratories are using. And as in human clinics, the achievement of reliable results requires strict compliance to standardized protocols and efficient method evaluation and quality management.

Quality assurance and management in clinical chemistry is essential for error identification and resolution at the pre-analytical, analytical, and post-analytical phases. Errors or "mistakes" are usually difficult to identify at both the pre- and post-analytical stages. Examples of these include mistakes in animal identification, inappropriate specimens, use of the wrong anticoagulant, inconsistencies in results between the analyzer & laboratory report, and loss of results. The analytical variability, on the other hand, is affected by imprecision and inaccuracy and can be monitored by following the internal quality control (IQC) module performed every day with the laboratory`s reagents and analyzer. This allows identification of random and systematic errors caused by expired reagents or calibrators, instrument failures, changes in analyzer`s measuring unit, among others. Besides IQC, another way to identify errors in clinical chemistry laboratory is by external quality assessment (EQA) schemes.

The Czech Center for Phenogenomics spearheaded the project aiming to analyze the IMPC data set to identify genes causing alterations in clinical blood chemistry profiles and can be translated to human disease. As a first step of this initiative, discussions were held to address data quality and management which are essential to improve data robustness. This includes data curation and inter-laboratory comparison trials, or ring trials. The latter involves the evaluation of the performance of diagnostic procedures using identical samples distributed in several laboratories. This is how repeatability and reproducibility of the assays can be assessed in the EQA.

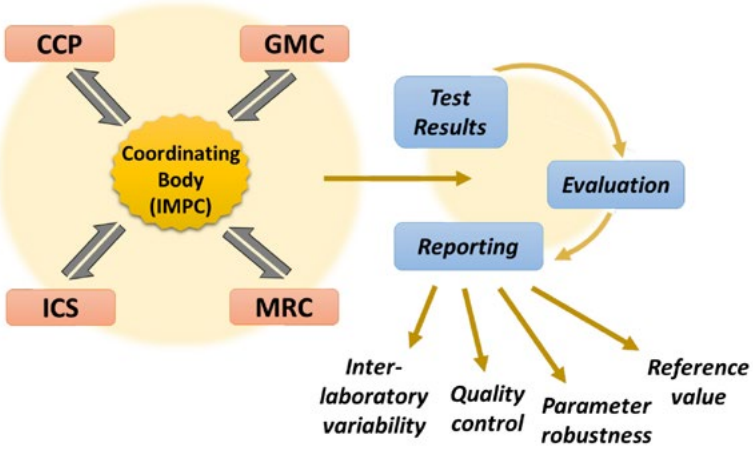

Figure 1: Schematic of the IMPC clinical chemistry ring trial.

Besides the CCP, other centers who participated in the ring trial are the German Mouse Clinic (Munich), MRC Harwell Institute (Oxford), and Institut Clinique de la Souris (Alsace) (Figure 1). The CCP was involved in the preparation and shipment of the pooled mouse plasmas that were used by other participating centers, who agreed upon common instructions for turn-around times, thawing and processing of samples. The results from all clinics were assimilated and analyzed by the IMPC data wranglers.

Figure 2 summarizes the variability of 22 clinical chemistry parameters measured in four batches of ring trial samples measured in May, June, and November 2019. This shows that across the four IMPC centers, some parameters display less repeatability than the others. This include lactate dehydrogenase, creatine kinase, amylase, total bilirubin and LDL cholesterol. The ring trial also enabled us to ascertain the intraclass correlation or agreement across the 4 centers (http://www.statstodo.com/IntraclassCorrelation_Pgm.php). For example, in the last batch of the ring trial, alkaline phosphatase measurements showed an excellent correlation (ICC >0.9) while LDL cholesterol displayed poor correlation (ICC <0.5) across the participating centers (Figure 3). Phosphorus, triglyceride, glucose, iron, total cholesterol, ALT, K, AST, urea, and total protein likewise gave an excellent inter-rater agreement coefficient. This shows that despite center differences in analyzers and investigators, the methods are characterized by repeatability, reproducibility, and robustness.

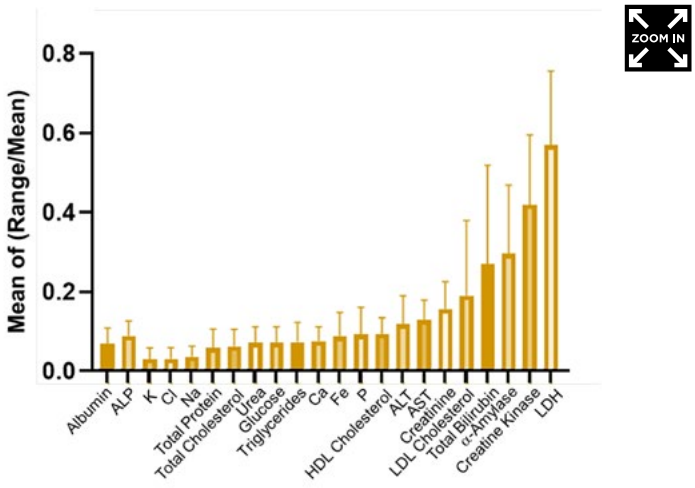

Figure 2: Variability of 22 clinical chemistry parameters measured in the ring trial.

From this ring trial, centers were also able to identify factors that contribute to the variability observed on particular parameters. For example, alpha-amylase results from CCP differed significantly most likely due to a difference in reagents. Besides variabilities and intraclass correlations in four centers, the analyses of clinical chemistry screens by the IMPC were extended to other centers and comprised of a compiled catalog with basic information on the 22 parameters. The extended analyses included comparison of equipment (manufacturer and model), anesthesia used dur-

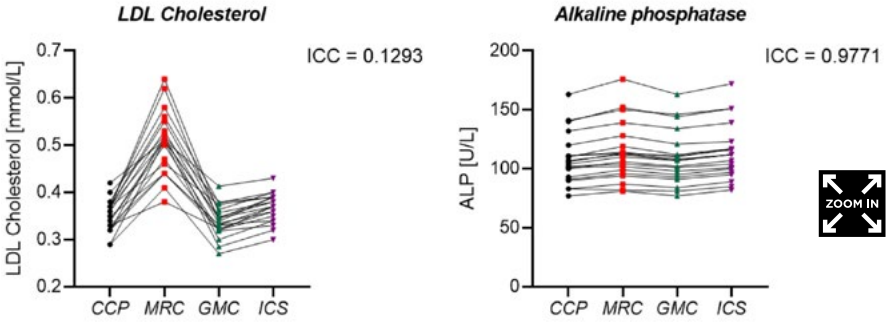

Figure 3: Comparison of LDL cholesterol and alkaline phosphatase measurements across the 4 IMPC centers. ICC: intraclass correlation coefficient

ing blood collection, method of blood collection, sample status (fresh or frozen), sample dilution, hemolysis status, anticoagulant used, storage temperature, and whether animals were difficult to bleed. All this information is available for both male and female wild type mice allowing identification of sexual dimorphism.

Currently, statistical analyses are being conducted by the IMPC data wranglers to handle outliers and summarize everything in tabular format. After this, reference ranges for each center and parameter are expected to be generated. This is one of the major objectives of this initiative which will benefit not only the member institutes of the IMPC but also scientists using mouse clinical chemistry in their research. As an ongoing further action, novel links to human diseases based on the complete clinical chemistry data in the IMPC will be established. Machine learning techniques will be utilized to detect deviations in clinical chemistry profiles of IMPC mutant lines that can be linked to human pathologies.

Acknowledgments: The authors acknowledge the valuable contributions of Tertius Hough & Sharon Cheng (MRC Harwell, UK), Birgit Rathkolb & Gregor Miller (GMC Helmholtz Center Munich, Germany), and Marie-France Champy (ICS Illkirch, France).

PRECLINICAL TESTING AND GLP IN CCP – CURRENT STATUS AND FUTURE AIMS

Sarka Suchanova

The Czech Centre for Phenogenomics can offer a very broad portfolio of highly standardized assays useful for preclinical studies on rodents including established disease models. Experiences from standardized phenotyping enable us to run very comprehensive experiments and thus be able to answer key questions for drug development. Furthermore, participating in the services of the Centre for Preclinical Testing (CPT, www.prekliniky.cz) we can offer usual preclinical tests under GLP regulations (Certificate of Good Laboratory Practice obtained in 2017).

We can plan and conduct projects addressing a broad variety of physiological categories from single parameter analysis (for example glucose level) to multi-parameter analyses using panels from clinical biochemistry, immunology, cytokine levels, etc. The preclinical pipeline can also be tailored according to customers' interests covering several physiological assessments from the whole CCP portfolio.

Preclinical tests currently implemented at the CCP

- Pharmacokinetics & pharmacodynamics & toxicity testing
- DSS induced colitis
- AOM-DSS model of colitis induced cancerogenesis
- High fat and high carbohydrate diets to induce obesity and diabetes
- CDX – cancerogenesis progress analyzed by whole-body imaging (luminiscence)
- Production of genetically modified mouse mutant models for preclinical testing (e.g. recently produced COVID 19 related models)

Good Laboratory Practice (GLP)

- GLP is a quality system related with the organisational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported.
- Ensures through careful and accurate documentation, covering all aspects of study and of its environment, the quality, integrity and reliability of test data.
- GLP is a requirement in regulatory, non-clinical, safety testing of the test items (pharmaceutical products, veterinary products, food additives, feed additives, industrial chemicals)

✔ Obtained data are accepted by receiving/regulatory authorities (SUKL in CZ –State Institute for Drug Control).

✔ Obtained data are reliable, reproducible and comparable between countries

- Other available services can be found on our websites https://www.phenogenomics.cz/preclinical-testing/

IMPLEMENTED TESTS UNDER GLP REGULATION

- The GLP mode is currently available for the histopathology and biochemistry & hematology units.
- Biochemistry & Haematology: terminal blood sampling under isoflurane anesthesia, whole and differential blood cell count, clinical biochemistry measurement using an automated analyzer, comprehensive statistical analysis of data

» Histopathology: comprehensive mouse necropsy including gross pathology and organ collection for further processing, complete tissue processing from fixation to staining, evaluation of tissue pathology, slide scanning, statistical analysis of data
» CCP is working under GLP regulation as a pilot testing site for three different GLP test facilities (one academic institution and two commercial companies)

COOPERATION WITH INDUSTRY

» The work of CCP and its service portfolio is very attractive for pro-profit organisations. Several companies are cooperating with CCP already, e.g. Sotio a. s., TSE Systems GmbH, Techniplast, Apigenex s.r.o., EXBIO, Biopharm a.s., Dyntec s.r.o., Infrafrontier GmbH.

OUR FUTURE AIMS

» To expand service portfolio for preclinical testing in GLP or GLP-like mode
» To extend GLP to the animal facility and other relevant CCP units.
» To maintain top-level quality of our services.
» To improve collaborations with academic institutions and industry to foster preclinical research.

LIPIDOMICS

Karel Chalupsky

WHAT IS A LIPID?

For chemists, a lipid is an ester of a carboxylic acid and an alcohol. Based on the composition, most lipids can be divided into fats, oils, waxes, phospholipids, and glycolipids. Fats and oils are esters of carboxylic acids with a carbon number of 16-20 (most often C16-C18) and glycerol. Glycerol has three alcohol groups and thus the variability of the lipid structure increases with not only the attachment of different carboxyl groups, but also their position on glycerol. Their main function is to store energy for the organism. Waxes are also esters of carboxylic acids most commonly with a C12-C18 carbon content, but instead of glycerol, they are bound to a single OH group in the aliphatic alcohol. Phospholipids consist of four parts, a fatty acid, an alcohol, phosphoric acid, and a modifier on phosphoric acid - choline, ethanolamine, or serine. Phospholipids are characterized by the bipolarity of the molecule, one part is polar and the other is non-polar. Their main occurrence and function is the formation of a bipolar cell membrane. Glycolipids also have a similar structure to phospholipids, where a carbohydrate is part of the molecule.

WHAT IS A LIPID GOOD FOR?

Lipids have three main functions in biological systems, the formation of membranes, energy storage and, in part, as signaling molecules. The formation of membranes is given by the bipolar characteristics of the phospholipid molecules of which they are composed - polar and non-polar part. The formation of the lipid bilayer is considered crucial to life, and probably its formation has helped to develop life on Earth. Nevertheless like any life, it needs a substance and energy exchange. This is the second important function of lipids. Their molecules are charged with energy, which the organism creates and releases as needed.They are therefore key molecules for the metabolism of organisms. As everything needs to be monitored and regulated, lipids have also this function. Lipids act as a signal transporter in the cell and their detailed role in the regulation of cellular processes is still emerging.

HOW LIPIDS ARE MEASURED?

The simple answer would be - as described above lipids are substances with a variable structure where their properties and function can change with the number and position of the double bond in the molecule. However, because lipids are incredibly interesting and important molecules, the whole Department of Metabolomics and Lipidomics deals with the detection and characterization of lipids. Thanks to the development

of mass spectrometry, chromatography and data processing, it is now possible to study and profile lipids in biological samples, leading to the discovery of new potential biomarkers among lipids. Lipid measurement begins with sample collection. Here, it is probably most important to avoid long incubations at room temperature, repeated freezing and thawing of samples, and exposure to lipase activity, thus to their degradation. The next step is the extraction of lipids. Lipids as described above are rather non-polar substances and have poor solubility in water. The most commonly used technique is liquid-liquid extraction using Folch, methyl-tertbutyl ether or butanol /methanol extraction. Each of these methods have their advantages and disadvantages. The biggest problem is cross-contamination of samples with polar phase, optimization of extraction for a specific class of lipids and their degradation. Chromatographic separation of lipids is crucial for detection by mass spectrometry. Some lipids differ very little and their separation is difficult. Thin-layer chromatography gas chromatography liquid chromatography (LC), high-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), capillary electrophoresis (CE), and two-dimensional (2D) techniques are used as separation methods. However, details and comparisons of these methods are beyond the scope of this article. Lipidomic data processing is similarly important. Very briefly, the most common is the comparative method of principal component analysis (PCA). As we usually cannot determine the exact chemical identity of lipid, including the location and number of double bonds, a common form of data presentation is discriminant methods, which attempt to differentiate the classes of lipids and find relationships between them. LipidSearch (Thermo Fisher Scientific) LipidHome and the LIPID MAPS consortium (www.lipidmaps.org/tools/index.html) are the most used lipid analysis software, but multiple programs and/or software packages are developed for lipid research.

LIPIDOMIC DATA IN THE BIOLOGY OF ORGANISMS

Logically, lipids are very important in studies and prediction of diseases related to the metabolic syndrome, which is a systemic disease typical of obesity, high blood pressure and diabetes. Kontush A, Lhomme M, Chapman M. Unraveling the complexities of the HDL lipidome. J Lipid Res. 2013; 54: 2950-63. In addition to prediction and diagnosis, lipids and their measurement are also used to monitor treatment. Neurological disorders are another goal of lipid studies because the brain contains the largest amount of lipids of all tissues. Here again, the focus is on early prediction and monitoring of conditions mainly associated with Alzheimer's Disease using differential lipid analysis. Grimm M, Mett J, Grimm H, Hartmann T. APP Function and Lipids: A Bidirectional Link. Front Mol Neurosci. 2017; 10: 63. The last most common pathophysiological condition where lipid measurements are common is cancer. Rapidly proliferating cells need to grow rapidly, their metabolic turnover is high, and lipids as signaling molecules are associated with migration, proliferation, and survival of cancer cells. As a result, cancer cells differ from healthy cells and thus their changes in lipid metabolism are very useful for diagnosis and therapeutic monitoring.
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Lipidomics is therefore part of metabolomics dealing with the analysis of lipids. Like other omics approaches, generating big data sets can be a challenge: data processing, the complexity of lipid structures, the number and position of double bonds, and the associated various physical properties. In organisms, there are thousands of chemical lipid entities that need to be isolated, separated, and identified. However, in recent years there has been great developments in separation methods and especially bioinformatics tools for lipid detection, which help us to constantly improve measurement capabilities and the quality of lipidomic data in biological systems.

Figure 1

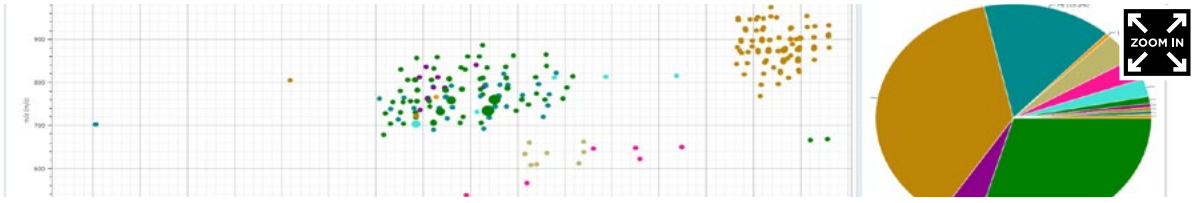
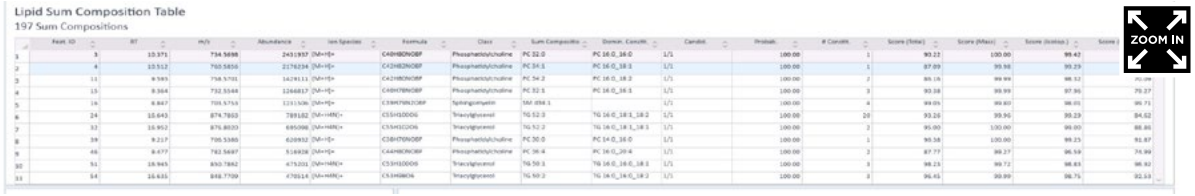


Figure 2



CCP OPEN CALL FOR COLLABORATIVE PROJECTS

The Czech Centre for Phenogenomics (CCP) will provide the services announced in this open call free of charge.

Eligibility: The call is open to researchers working for academic institutions and conducting independent/basic research, e.g. universities and research institutes. Both Czech and international applicants are welcome (EU or non-EU). This call is not intended for companies.

GENE NOMINATION & PRIMARY PHENOTYPING

- Applicants select an available gene for KO mouse line production from the iMits database.
- CCP will provides first-line phenotyping service free of charge.
- In total, ten mutant lines will be phenotyped.

SECONDARY PHENOTYPING / LINE DISTRIBUTION

- Applicants select one mouse line from the first-line phenotyping resource generated by the CCP.
- Applicants may apply for:
  1) Secondary phenotyping of the selected mice line
  or
  2) Line distribution.
- In total, three projects for secondary phenotyping and three projects for line distribution will be performed free of charge.

For more information visit the CCP website
www.phenogenomics.cz/about-us/open-calls

CENTRE FOR PRECLINICAL TESTING - SERVICES ON PRECLINICAL TESTING

After obtaining the certificate of Good Laboratory Practice (GLP) in January 2017, the Centre for Preclinical Testing (CPT) commenced activities with the support of the Czech Academy of Science (CAS). The primary mission of the CPT is to perform preclinical testing of substances that have successfully passed through basic research, and thus to contribute towards the development of new pharmaceuticals to combat life-threatening diseases, including those currently difficult to cure.

The Institute of Physiology CAS performs a coordination role for the CPT, and testing is also performed by the Institute of Molecular Genetics CAS including National infrastructure CCP, the Institute of Animal Physiology and Genetics CAS, and the Institute of Biotechnology CAS.

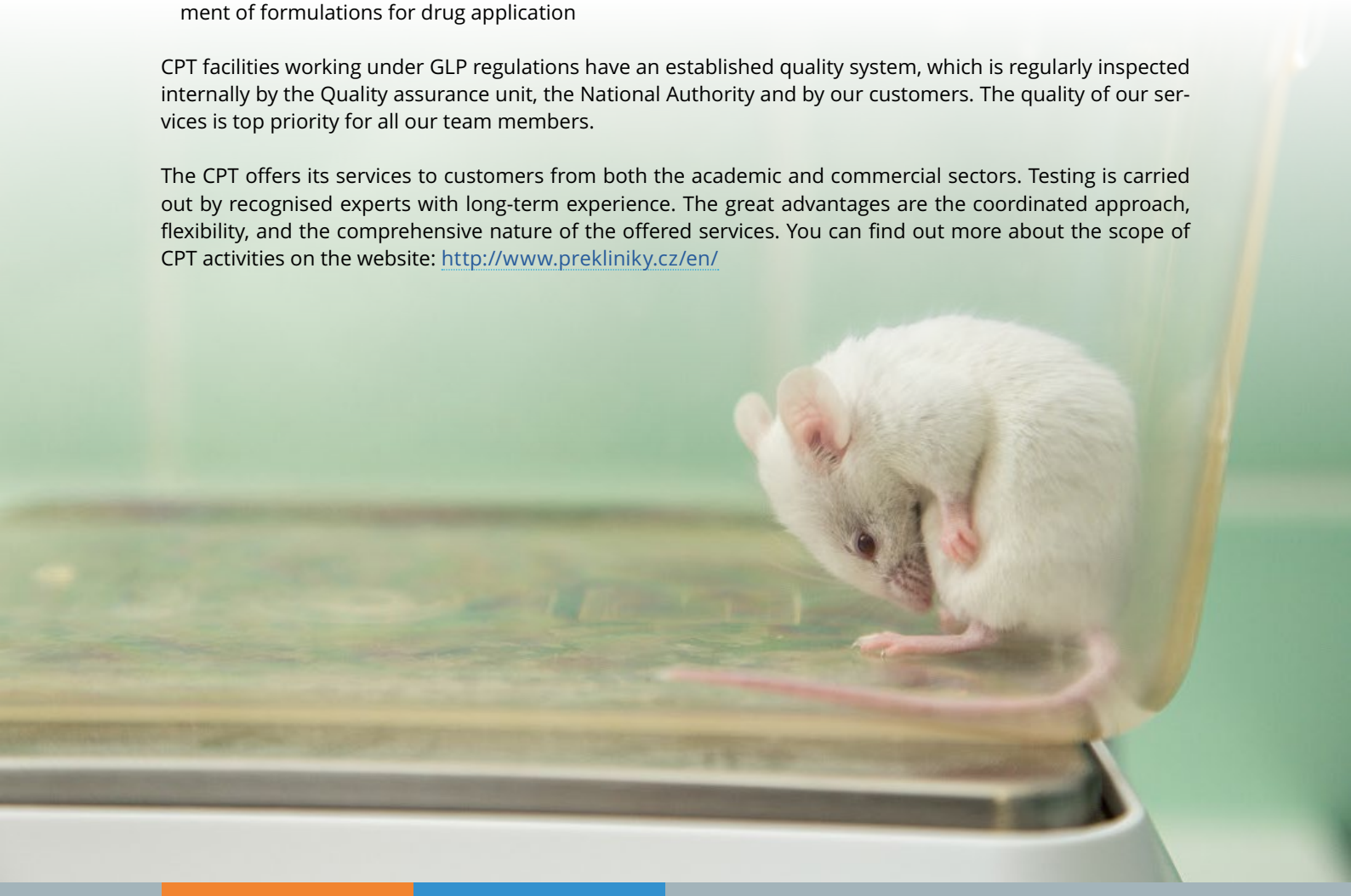
The CPT offers a broad portfolio of tests under Good Laboratory Practice (GLP).

CORE SERVICES:

- **Toxicity studies,** including toxicokinetic studies of promising chemical or biological agents on model animals – rodents and non-rodents (test systems: mouse, nude mouse, rat, guinea pig, rabbit, minipig) in compliance with ICH and OECD guidelines.
- **Bioanalytical, hematological and biochemical testing** of samples taken from animals during toxicity studies (determination of active substance in plasma or other biological matrices).
- **Development and validation of bioanalytical methods** for various test systems and biological matrices.
- **Determination of metabolites** in tissues and biological matrices (blood, plasma, urine)
- **Histopathological evaluation** of tissues from animals used in toxicity studies
- **Pharmacological studies on xenografts** (nude mice) with various cancerous cell lines, including Patient Derived Xenografts (PDX)
- **Cardiology diagnostic tests on animal models** – electrocardiogram (ECG), blood pressure measurement, cardiac imaging (Echo)
- **Synthesis, characterization and certification** of chemical substances with therapeutic potential, development of formulations for drug application

CPT facilities working under GLP regulations have an established quality system, which is regularly inspected internally by the Quality assurance unit, the National Authority and by our customers. The quality of our services is top priority for all our team members.

The CPT offers its services to customers from both the academic and commercial sectors. Testing is carried out by recognised experts with long-term experience. The great advantages are the coordinated approach, flexibility, and the comprehensive nature of the offered services. You can find out more about the scope of CPT activities on the website: http://www.prekliniky.cz/en/



CZECH CENTRE FOR PHENOGENOMICS

The Czech Centre for Phenogenomics (CCP) is a large research infrastructure unique in combining genetic engineering capabilities, advanced phenotyping and imaging modalities, SPF animal housing and husbandry, as well as cryopreservation and archiving, all in one central location – at BIOCEV campus.
Through its membership in INFRAFRONTIER and IMPC, CCP is a partner in a global network that aims to comprehensively and systematically analyze the effect of loss of function gene mutations in mice. The goal is to produce a comprehensive 'encyclopaedia' of gene function, that will help identify causative factors of human diseases as well as novel targets for therapeutic intervention.

www.phenogenomics.cz

Czech Centre for Phenogenomics

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MINISTRY OF EDUCATION, YOUTH AND SPORTS

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EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research, Development and Education

Operational Programme Research, Development and Education (OP RDE)

- CZ.02.1.01/0.0/0.0/16_013/0001789 Upgrade of the Czech Centre for Phenogenomics: developing towards translation research
- CZ.02.1.01/0.0/0.0/18_046/0015861 - Infrastructure upgrade of CCP II

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