Foundation for Polish Science

TEAM TECH PROGRAMME

LAUREATE ANNUAL RESEARCH PROGRESS REPORT

Project title:	**Mass Spectrometry of Biopharmaceuticals - improved methodologies for qualitative, quantitative and structural characterization of drugs, proteinaceous drug targets and diagnostic molecules.**		
Laureate:	**Prof. Michał Dadlez**		
Reporting period:	From 01.10.2019 to 30.09.2020	**Period no.**	**3**
Agreement No.:	TEAM TECH CORE FACILITY/2016-2/2	from 01.11.2017 to 31.10.2020	

NOTE: Information provided within a progress report shall consider only the said project within a particular reporting period.

1. INFORMATION ON THE RESEARCH PROGRESS

1.1. Achieved deliverables and milestones (up to 1 page A4)

List up to 3 (three) the most important research achievements within a past reporting period:

1. Completed development of the 2D-Micro-Nano-LC fractionation using 96-well-plate microliter fraction collection and a PAL System robot with the resulting increase in sensitivity (7-10 fold) opening the possibility to targeted analysis of low quantity samples (60ug of tissue biopsy)
2. Development of a immunotherapeutic analysis toolbox, including multiplexed Multiple Reaction Monitoring (MRM) LC-MS method, capable of accurately quantitating Pertuzumab (Perjeta), Rituximab (Rituxan), and Trastuzumab (Herceptin) in human plasma using multiple specific peptides in a single analysis, native MS analysis and HDX pattern structure characterisation.
3.Optimized LC-MS/MS methods for a panel of neurotransmitters, of FAS (fetal alcoholic syndrome) markers; trimethylamine derivatization method, enabling LC-MS/MS analyses in complex matrices using new sample prep protocol (plasma, urine and stool extract)

Provide a brief and concise summary of the above mentioned achievements, indicating also impact of such deliverables/milestones on the overall project progress:

Deliverables mentioned complete or fulfill in part the aims of relevant tasks planned in the project at the present stage.

a. Project challenges and risk assessment (up to 1 page A4)

List up to 3 (three) the most important project and research challenges experienced within a given reporting period supplemented with a brief and concise information on the actions taken:

Pandemic blocked normal communication channels and broken supply chains to some extent, substitute measures were being taken, using internet communication, and alternative sources of consummables etc. Due to that difficulties met do not exceed the routine level of scientific conduct and are of technical or merit-based nature.

- […]
- […]

Provide a brief and concise information on the obstacles in the project implementation - research and managerial – that are likely to occur within a forthcoming reporting period and your strategy to respond and mitigate:

[…] No obstacles reported.

b. Socio-economic impact of the project and its tech-transfer potential (up to 1 page A4)

Provide information on how the results achieved during the project implementation influence the key scientific, technological and socio-economic challenges of the modern world.

Indicate any actions taken in the past reporting period that may help facilitate a tech-transfer, including collaboration with identified economic partners.

New tissue homogenization methods developed within the CORE grant allowed us to sign a contract with FiLeClo company for multiplexed drug analysis in a wide distribution study in 243 tissue samples (brain, kidney, heart, lungs, liver and adipose tissue). Established in the lab procedures for drug and metabolites monitoring are directly transferred to application for medical purposes by biotech, biopharma companies like pharmacodynamics studies of new drug candidates for OncoArendi, carried out by the Lab. Proteogenomic approaches applied during our collaboration with dr hab. Urszula Zielenkiewicz in her work with bacterial consortia may pave the way for efficient remediation of plant wastewaters. New bioinformatic procedures for detection of non-canonical peptide sequences will be used in a common project with a company “Kucharczyk – techniki elektroforetyczne”.

c. Detailed description of the research activities (up to 6 pages A4)

This section shall provide a thorough information on the progress of the research programme in the past reporting period. In case of consortium, sections shall be sub-divided between two members of the consortium.

In the third reporting period the implementation of the new analytical procedures was continued as planned. In the frame of all four tasks of the project numerous procedures were designed, tested

using pilot sample sets according to appropriate procedures. SOPs were worked out and validation procedures carried out when necessary.

For targeted proteomics:

Development of new MRM/PRM technologies to expand the assay service portfolio: To increase the detection of low level proteins such as phosphoproteins and cancer markers in MRM/PRM analysis in limited-quantity biological samples we have completed development of the 2D-Micro-Nano-LC fractionation. The high-pH reversed-phase fractionation method with 96-well-plate microliter fraction collection using a PAL System robot was developed in-house as no such devices are commercially yet available. The columns, specific materials for capillaries, and the buffer system were optimized for system stability. As a proof-of-concept, the resulting increase in sensitivity (7-10 fold) allows to quantitate 18 low abundance cell-cycle control proteins (e.g. CDKs/cyclins) out of a 25-MRM-protein panel, versus 11 proteins with a direct MRM analysis, in only 60 ug of a cancer cell-line sample. This approach will allow to provide the ability to offer new services in cases of small amounts of samples such as e.g. cancer biopsies that could not be effectively analysed before by targeted approaches.

Development of new MRM/PRM protein assays and expansions of existing panels: In the last period, the following new multiple reaction monitoring (MRM) assays and panels have been developed. We have developed a 9-protein MRM panel for the quantitation of the important ribosomal protein components (Rpl40, Rpl17, Rpl17A, Rpl15, Rps29A, Rpl34, Rps26, Rpl39, Rpl10) using 24 SIS peptides and an optimized sample preparation method, and applied it for the study of ribosomal structures in collaboration with Dr. Ulrike Topf (IBB-PAS). We have finalized a multiplexed cytokeratin (CK) MRM panel composed of 18 CKs (CK1, CK2, CK3, CK5, CK6, CK6A, CK6B, CK9, CK10, CK14, CK15, CK16, CK17, CK23, CK77, CK78, CK79 and CK80), and a set of 4 control proteins, employing 45 SIS peptides. This optimized 1 hour-long NanoLC-MRM-MS analysis was applied in collaboration with Dr. Katarzyna Wertheim-Tysarowska from The Institute of Mother and Child in Warsaw in a study of epidermal pathologies in 46 children, revealing the epidermal CK profile in comparison to genetic and metabolomics information. Finally, towards developments in the targeted quantitation of phosphoproteins, we have developed MRM and Parallel Reaction Monitoring (PRM) methods for the specific quantitation of a phosphorylation site on the GRP8 (Glycine-rich RNA-binding protein 8) protein to study stress responses in *Arabidopsis thaliana* by Dr. Grazyna Dobrowolska (IBB-PAS).

Development of targeted MS assays for therapeutic monoclonal antibodies: In the last period we developed a targeted proteomics method for the therapeutic drug monitoring of three monoclonal antibodies (mAb) using a multiplexed targeted mass spectrometry (MS)-based assay with internal peptide standards. Specific and accurate quantitation of mAb drugs is required for therapeutic drug monitoring (TDM) and for personalized dosing. We developed a multiplexed Multiple Reaction Monitoring (MRM) LC-MS method capable of accurately quantitating Pertuzumab (Perjeta), Rituximab (Rituxan), and Trastuzumab (Herceptin) in human plasma using multiple specific peptides in a single analysis. The method uses an external mAb calibration curve and internal stable isotope-labeled standard (SIS) peptides (^{13}C,^{15}N-C-term-K/R) for increased sensitivity, specificity and absolute quantitation. For absolute specificity, unique peptides within the variable light and heavy chain

regions, including the Complementarity-Determining Regions (CDRs) were selected in each antibody. The multiplexed 1h nanoLC-MS analysis quantitates the three mAbs with 20 SIS, and additionally measures total IgG1, IgG2, IgG3, and IgG4 antibody levels with 8 SIS for an additional level of medical information. Furthermore, five digestion control plasma proteins are monitored to ensure assay robustness. The digestion step eliminates potential interferences from anti-drug antibodies (ADAs) and avoids the many issues of ELISA-based TDM regarding specificity and accuracy. We optimized the detergent-aided (NaDOC) sample preparation step for maximal sensitivity. The method requires less than 10 uL of plasma and accurately quantitates the three mAbs over their expected therapeutic range from 10 to 500 ug/mL. This is done directly from plasma without requiring depletion or enrichment techniques, while measuring multiple peptides per mAb for increased accuracy. A limit of quantitation (LOQ) of 10 ug/mL, with an accuracy and precision (%CV) within 20%, was achieved for 2-4 peptides per mAb, with a limit of detection (LOD) reaching as low as 350 ng/mL. The method now awaits validation on patient samples in collaboration with the University of Bialystok. This development demonstrates that analogous TDM assays can be made by us for other popular mAbs. Such TDM by MRM can guide physicians on dosage adjustment for individual patients to optimize the response to therapy, while lowering costs and reducing side effects.

The protocol for Native-MS analysis of antibodies has been worked out along with Standard Operating Procedure. This allows for the analysis of homogeneity of a given preparation of immunotherapeutic antibodies and the pattern of posttranslational modifications, the basic parameters characterising the protein biosimilars.

For HDex protocols in MS based MAbs analysis toolobox the analyses in the reported period focused on HDX-MS exchange patterns in Herceptin. The best HDX-MS conditions for this protein were experimentally selected. The concentration of Herceptin was 55 mg/ml. This concentration was chosen because Herceptin is sold as a powder to obtain 21 mg/ml and 120 mg/ml final solutions. The primary sequence coverage upon PLGS processing and DynamX filtering was significantly improved with optimised protocol (95,4% for heavy chain and 98.1% for light chain), but after all data processing and envelope analysis 81.3 % for Herceptin light chain and 81.4% for heavy chain sequence coverage was obtained. Especially variable region (VR) of a light chain was poorly covered. 5 time points of exchange were analysed - 10sec, 1 min, 5 min, 30 min and 2.5 hr. The proper controls of maximum and minimum exchange were also done. For heavy chain, 10 sec of D2O exchange revealed moderate protection almost for the whole fragment with some exceptions. Only the N-terminal part of a hinge region and CDR3 are almost fully exchanged, whereas some parts of constant region (CR) show very high protection, even at longer incubation time with deuterated buffer. For the light chain, the protection is higher, especially in constant region, which fragments remained not fully exchanged even after 150 min of deuterium exchange. A benchmarking exchange pattern, characteristic for this drug 3D structure was thus established. These results provide a sound basis as a template for comparison of the 3D structure in biosimilar products. We were however not able to purchase any biosimilar of Herceptin (like Herzuma or Kanjiti) to do such comparison due to poor availability of such biosimilar on polish market.

The three procedures of the analysis of antibodies, described above, constitute a complete toolbox for comprehensive analysis of immunotherapeutic drugs by MS. This new service in the laboratory greatly enhances its repertoire of medically oriented service, its application for use in the clinic is in progress in cooperation with collaboration with the University of Bialystok.

Within the subproject focusing on HDex data analysis procedures HaDeX - an in house tool for HDX data analysis - was improved. Analytical workflow of the HDX-MS data subjected to analysis by HaDeX has been critically scrutinized also supported by literature research and confronted with known approaches to presenting the results of the experiment. This allowed to incorporate a more universal way of conduct. Conclusions were incorporated into HaDeX. HaDeX is routinely used for the analysis of different protein assemblies. In the last period a large set of experiments on Ssq1/Isu1/Jac1 complex was performed in collaboration with prof. Jarosław Marszałek research group from University of Gdansk. The investigated *Saccharomyces cerevisiae* complex is indispensable for iron-sulfur cluster biogenesis, which appears to be an essential function for mitochondria. Also a set of experiments on CHN1 and Ufd2 proteins were performed as part of collaboration with prof. Wojciech Pokrzywa research group from International Institute of Molecular and Cell Biology, Polish Academy of Sciences. CHN1 and Ufd2 are *Caenorhabditis elegans* proteins involved in the pathway of protein ubiquitination. The aim of these experiments was to establish the possible interaction sites in the investigated complexes. Two Rab proteins and Rab Escort Protein (REP) were studied by HDX for contact sites identification in collaboration with prof. L. Surmacz, and the manuscript has been prepared, where we identified the interaction interfaces between Rab and Rep proteins. Such “real-life” datasets and direct contact with the users provided fast feedback from the researchers on new features that would be needed in such a data analysis tool, and currently missing in the other existing tools. This resulted in addition of new features to HaDeX (e.q tooltips, helpers, etc), and improved documentation portfolio. New features were fast tested on next sample sets after each update. Improvements of HaDeX resulted in successful publication of an article in Bioinformatics.

For global proteomics procedures:

Cysteine reduction and alkylation is a critically important step in preparing samples for global proteomic analysis influencing the final output of properly sequenced peptides. Blocking the cysteines prevents the formation and reshuffling of disulfide bridges. Their formation significantly increases the complexity of the mass spectra. In spite of the importance of this step there is no consensus among the research groups and in the literature on which blocking reagent is the most effective. To our knowledge decisive tests are not available in the literature, while different reagents in different reaction conditions are used in spite of common knowledge that in addition to blocking cysteines, they may react nonspecifically with other amino acid residues. Therefore for optimum results it is still of value to study the issue in a statistically valid experimental setup.
Evaluation was carried out for three standard methods of sample preparation: in-solution digestion, filter-aided sample preparation (FASP) and on-bead digestion SP3. All assays were performed on commercially available HeLa cell protein extract, which is the most widely used standard in proteomic core facilities. Additionally, for the FASP method, experiments were also carried out on a human plasma sample due to the high content of thiols in this material. The cysteine reduction for all samples was performed using the TCEP reagent due to its high efficiency and stability. Three cysteine blocking agents were tested: iodoacetamide (IAA), chloroacetamide (CAA) and methyl methanethiosulfonate (MMTS). All tests were carried out in triplicate on QExactive mass spectrometer. Data was analysed using the MaxQuant / Perseus package with cysteine-specific and off-target variable modifications: carbamidomethylation (for IAA and CAA) and methylthio (for MMTS

samples). Dependent peptide search was performed to discover other, non-defined modifications, triggered by the blocking reagents.

Although all in-solution tests used the same amount of HeLa extract, most peptides and proteins have been identified for the samples prepared using MMTS. The highest specificity of cysteine modification was also observed for this reagent, with the lowest amount of off-site modifications and missed cleavages. Such results are surprising, as MMTS is not a commonly used and well described reagent, due to the theoretical reversibility of its reaction with cysteine.

Despite the widespread use of IAA in proteomics, a disturbingly high number of off-site modifications was observed, reaching as much as 50% for the K, H (ca) D, E, S, T, Y and M residues. Additionally, iodoacetamide caused modification of lysine imitating the mass of GlyGly modification, which categorically excludes its use in ubiquitination studies. Chloroacetamide gave 20% less identifications than MMTS, with almost 10-fold lower number of off-site modifications compared to IAA. In studies where cysteine carbamidomethylation is desired rather than methylthio modification, CAA will be a better choice than IAA. In the FASP and SP3 experiments, the differences between the reagents were not so substantial, but still spoke in favour of MMTS. A slightly different situation was observed for the FASP digested plasma, where the highest number of identifications was achieved for the samples prepared with IAA. This observation may be related to the large population of thiols in the plasma, which affects the equilibrium of the reaction between MMTS and cysteine residues.

Based on the conducted experiments, the use of MMTS was recommended for the further proteomic studies, as these reagents cause the least non-specific modifications. Plasma samples or similar body fluids are the rare exception, where the use of chloroacetamide might be recommended. IAA usage should therefore be discouraged.

In the reported period three new articles presenting results of global proteomic approaches were published. For the paper by Bryk et al. we established methods of quantifying glycated tryptic peptides of antiplasmin. Publication Kotrys et al. is presented implementation of isobaric labelling for identification and quantification of subcellular structures, in this case mitochondria. Implementation of the proteogenomic approach in collaboration with Dr. Urszula Zielenkiewicz allowed for the global proteomics of bacterial metaconsortia present in the wastewater plant connected with the sugar factory. The bioinformatics workflow was worked out allowing to connect the reference databases of bacterial and archea protein sequences data from the metagenomic DNA sequencing plant, allowing to obtain a collection richer in protein variants specifically present in this system. For these demanding set of samples specific sample prep protocols were worked out resulting, when combined with newly created sequence databases, in identification of crucial proteins responsible for bioremediation in plant waste environment.

Within Task 4 new procedures for quantitation of low molecular compounds were established and tested as planned.

An optimized LC-MS/MS method for quantitative analysis of a panel of neurotransmitters in brain tissue homogenates was worked out, enabled by tissue homogenisation procedures obtained in frame of the CORE project in the previous periods and supported by the purchase of appropriate instrumentation covered by CORE. Its first application in neurobiological research is in the project lead by dr hab. Adam Hamed's project, Nencki Institute of Experimental Biology. We have also worked out a new approach to trimethylamine quantitation in biological matrices, supported by derivatization, which improved sensitivity of the assay. The sample preparation scheme was based on derivatization using tert-butyl bromoacetate. The protocol was tested and used for biological

samples analysed by prof. Marcin Ufnal (The Medical University of Warsaw) and dr hab. Robert Olek (Gdansk University of Physical Education and Sport). The third aim was the FAS markers detection in meconium: the ethyl esters, ethyl glucuronide and ethyl sulfate. The worked out protocol of sample prep will be optimised for LC-MS. This task will be implemented first in the project of dr Ewa Głuszczak-Idziakowska and prof. dr hab. Bożena Kociszewska-Najman (The Medical University of Warsaw). Also, SCFAs method, free and total L-carnitine method, amino acids derivatization using AccQ•Tag Ultra Derivatization Kit and dansyl chloride for amino acids and their metabolites quantitation have been worked out. SOPs (Standard Operating Procedure) for new methods are being worked out.

The panel consisting of 8 amino acids and 8 monoamines was optimised for most efficient extraction of those analytes during homogenization process of brain samples using homogenizer bought in frame of the CORE project. An LC-MS/MS method for quantification of 14 different modified nucleosides was also worked out using our systems, with establishing calibration curves to see whether the apparatus enables analysis of low concentration desired by the client.

Developed in previous period tissue homogenization protocol allowed us to take part in tender procedure issued by drug development company FiLeClo offering multiplexed drug analysis as a package. In frame of this contract the protocol was used in this reporting period for a wide distribution study of three compounds in a set of 98 blood samples and 243 tissue samples (brain, kidney, heart, lungs, liver and adipose tissue). Therefore the protocol matured and allowed us to increase the offer of the lab by distribution analysis in all organs which greatly increases our competitiveness. Another procedure that was included into the portfolio was the analysis of chemical stability.

1. PROJECT PROMOTION AND DISSEMINATION OF THE RESULTS

COVID pandemic state does not allow for most effective promotion by personal contacts and participation in scientific events. These activities were largely stalled starting march 2020. Nevertheless, several posters were presented on a few scientific conferences: Gottingen Oct, 2019, Warsaw Sept 2020, as reported in project students reports. Several scientific papers were published in collaboration as result of that project.

Instead, other means of communication, mails, web page, personal contacts, social media served to disseminate the knowledge on new possibilities offered thanks to CORE financing and gained new clients from BioPharma companies (FiLeClo, Selvita S.A., PureBiologics S.A., and scientific institutions (prof. Marszałek from Gdańsk Univ., prof. Pokrzywa IIMCB, Warsaw, prof. Chacińska, CENT, Warsaw, prof. Rodziewicz-Motowidło, U.Gd., Prof. F. Sobott, Leeds Univ.)

2. PARTNERSHIP IN THE PROJECT

d. Description of collaboration with foreign research partner(s)

This section shall provide a brief and concise information on the nature of collaboration with foreign research partner.

In the frame of CORE new procedures worked out streamlined several projects carried out in frame of long-lasting international collaborations, for instance in the areas of structural studies of proteins by MS, leading to a series of new publications with prof. prof. D. Glover, Caltech, Cambridge Univ., B. Negrutskii, MMCB, Kiev, Z. Domiński, North Carolina Univ., H. Herrmann, DKFZ Heidelberg, and sparked new collaborative efforts like with prof. F. Sobott, Leeds Univ., dr. M. Kulma, Slovenian Acad. Sci. These developments increased the lab impact on the European level, followed by the nomination of MS Lab IBB PAS as a coordinator in INFRAIA programme (under consideration) of a integrative structural MS project with a group of leading structural MS specialists, like A. Sinz, Germany, F. Sobott, UK, R. Grandori, Italy, S. Cianferani, Italy, J. Langner, Germany, J. Marcoux, France.
Metabolomic analyses of absorption of Polyunsaturated Fatty Acid (PUFA) in Neonatal Pigs were carried out in a common project with prof S. Pierzynowski, Lund Univ. Sweden. New proteomic approaches are used in Zika virus capsid-protein interactome co-immunoprecipitation experiments are performed with two teams lead by prof. Michał Hetman and prof. Dong Chung form Kentucky University.

e. Description of collaboration with local research partner(s)

This section shall provide a brief and concise information on the nature of collaboration with local research partner.

The Lab continues numerous long-lasting medically oriented collaborators (prof. Ufnal, prof. Pączek, Prof. Mucha, prof. Ostrowski, prof. Wielgoś, prof. R. Smoleński, prof. K. Koziak, prof. S. Jóźwiak, dr Ewa Głuszczak-Idziakowska and prof. dr hab. Bożena Kociszewska-Najman (The Medical University of Warsaw, dr. Katarzyna Wertheim-Tysarowska, etc.) but also numerous basic science groups.

from The Institute of Mother and Child in Warsaw applied a multiplexed cytokeratin (CK) MRM panel in a study of epidermal pathologies in children. Matabolomics analyses helped Prof. L. Rudnicka from Department of Dermatology, Medical University of Warsaw to elucidate the clinical implications of intestinal barrier damage in psoriasis. Dr Ulrike Topf, IBB PAS used a panel of ribosomal proteins to study regulation of protein homeostasis upon mitochondrial stress while prof. G. Dobrowolska, IBB PAS used Parallel Reaction Monitoring (PRM) methods for the specific quantitation of a phosphorylation site on the GRP8 (Glycine-rich RNA-binding protein 8) protein to study stress responses in *Arabidopsis thaliana*. Tissue homogenization procedures raised interest and collaborations with dr hab. Adam Hamed, Nencki Institute of Experimental Biology leading to a common work on neurotransmitters, with prof. Ufnal from Warsaw Medical Unversity on analyses of trimethylamine distribution, while with dr hab. Robert Olek (Gdansk University of Physical Education and Sport) on amino acid quantitation. New HDex approaches generated new collaborations on protein-protein interaction networks with: prof. Marszałek, U.Gd., prof. Pokrzywa, IIMCB, Warsaw and dr L. Surmacz, IBB PAS - on role of Rab Escort Protein (REP) on REP-Rab interactions in plant fertility.

3. IMPACT OF THE PROGRAMME ON THE PI's CAREER

Provide information and necessary feedback on how is this programme affecting your scientific and professional development.

Project enables transfer of research into new analytical procedures made available for a wider scientific community, but also provides new potential diagnostic tools of importance to medicine. Therefore it enables to disseminate the developments in a science oriented

environment into the society. It ensures a broader perspective for the activity of the Mass Spectrometry Lab, I have a privilege to direct. Project increased visibility and recognition of the Lab on the European level with the nomination of the project leader as a coordinator of the structural community networking and integration project submitted for INFRAIA Eu programme.

4. DEVELOPMENT OF RESEARCH TEAM MEMBERS

f. Information on the supervision of PhD students, including additional mentors

Since the aim of the project is to increase the human potential of the R&D sector, provide necessary information on the scientific and professional supervision of PhD students participating in the projects. It is crucial to provide information on mandatory supervision of PhD students by external mentors.

All students are supervised on a daily basis by the PI and postdocs due to pandemic state by remote conferences and mail correspondence. Lab meetings had to be temporarily discontinued. External mentors for PhD students are in contact with PhD students: Prof. J. Pierzynowski and prof. H. Herrmann, they actively participate in common projects in which both PhD students are involved. However, the formal acceptance of prof. Herrmann has not been obtained yet. Dr Michał Burdukiewicz (Warsaw Tehcnical Univ.) is mentoring PhD student working in structural proteomics field in aspects of statistics and programing.

g. Supervision of research staff

Since the aim of the project is to increase the human potential of the R&D sector, provide necessary information on the scientific and professional supervision of research team members.

All students and lab members are supervised on a daily basis by the PI and postdocs in mail and remote conferences and consultations. Lab meetings, held on a weekly basis, had to be discontinued due to pandemia, they will be reinstated as soon as the situation permits. Meeting are held by communicator platforms and all discussion held by mails.

5. IMPLEMENTATION OF THE PROJECT ACCORDING TO THE PLANNED SCHEDULE

YES X NO ☐

In case an actual implementation of the project differs from the accepted schedule, please do provide the rationale behind it:

No deviation from schedule.

6. ADDITIONAL INFORMATION

Provide – if necessary – all other important information relevant to the project implementation.

[…]

7. PI's WORKLOAD

I, the undersigned, hereby state that:

X☐ My total commitment to projects financed from EU Structural Funds, ESF and other activities financed from other sources, including the beneficiary's and other entity's funds, did not exceed 276 hours in any month from the reporting period.

☐ My total commitment to projects financed from EU Structural Funds, ESF and other activities financed from other sources, including the beneficiary's and other entity's funds, exceed 276 hours in the below indicated months from the reporting period*:

Year/Month	Number of hours of the total commitment per month
Oct. 2019- Sep.2020	220

8. TEAM MEMBERS WORKLOAD:

X☐ According to the team member's statements in no case the total commitment to projects financed from EU Structural Funds, ESF and other activities financed from other sources, including the beneficiary's and other entity's funds, did not exceed 276 hours in any month from the reporting period.

☐ According to the team member's statements in the following cases the total commitment to projects financed from EU Structural Funds, ESF and other activities financed from other sources, including the beneficiary's and other entity's funds, exceeded 276 hours in the below mentioned months from the reporting period*:

Name/Surname	Year/Month	Number of hours of the total commitment per month
Dominik Cysewski	2019.10-2020.09	170
Radosław Jaźwiec	2019.10-2020.09	170

Katarzyna Dąbrowska	2019.10-2020.09	170
Weronika Puchała	2019.10-2020.09	170
Emilia Samborowska	2019.10-2020.09	170
Mariusz Radkiewicz	2019.10-2020.09	170
Magda Kaus-Drobek	2019.10-2020.09	170

* In case of exceeding 276 hours monthly of the total commitment to any projects of an employee/stipendee, his/her remuneration/stipend from the Project funds will constitute an ineligible expenditure for each month in which 276 hours have been exceeded.

9. Appendixes to the merit-based annual progress report in the electronic version:

- Project realization indicators (on-line data base: *Progress reports* tab/ *progress report*/*Indicators*)
- Scientific Achievements of the Laureate and Team Members (on-line data base: *Progress reports* tab/ Publications/Patents/Academic degrees and titles/Awards and grants),
- List of conferences, scientific exchanges and business meetings (doc. file uploaded to on-line data base: *Progress reports* tab/*Progress report/Attachments*)
- Progress reports of team members – if applicable - (doc. file uploaded to on-line data base: *Progress reports* tab/*Progress report/Attachments*)

Note: The Laurate's Annual Research Progress Report should be uploaded to the Foundation's database (in the *Progress reports tab/Progress report/Attachments tab*) in two formats: as a '.doc' file and its signed, scanned version as a '.pdf' file.

I, the undersigned, hereby confirm that the information contained in the merit, periodic report (both electronic and paper version) are true. I am aware of the legal consequences of giving untrue information in a legally significant situation, as stated in article 271 of the Penal Code.

Date:……25.10.2020……

Foundation for Polish Science

Laureate Signature.....

European Funds Smart Growth

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