Expert Review of Proteomics

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ieru20

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To cite this article: Suresh Jivan Gadher , Piotr Widlak & Hana Kovarova (2020): Power of proteomics and progress in precision medicine – 13th central and eastern European proteomic conference (CEEPC), Ustroń, Poland, Expert Review of Proteomics, DOI: 10.1080/14789450.2020.1779065

To link to this article: https://doi.org/10.1080/14789450.2020.1779065

Accepted author version posted online: 08 Jun 2020.
Published online: 24 Jun 2020.

EXPERT REVIEW OF PROTEOMICS
https://doi.org/10.1080/14789450.2020.1779065

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MEETING REPORT

Power of proteomics and progress in precision medicine – 13th central and eastern European proteomic conference (CEEPC), Ustroń, Poland

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ABSTRACT

Introduction: Central and Eastern European Proteomic Conference (CEEPC) provides a platform for researchers to discuss multi-disciplinary integrated approaches to address a range of challenges from present day viral pandemic to on-going progress in Precision Medicine. CEEPC brings together various multi-omics entwined with novel enabling technologies, thus facilitating conceptual advances from cell to society for the benefit of mankind.

Areas covered: Proteomic methodologies, databases and software has revolutionized our ability to assess protein interactions and cellular changes, allowing the establishment of biological connections and identification of important cellular regulatory proteins and pathways previously unknown or not fully understood. Additionally, Mass spectrometry (MS) remains a major driving force in the field of 'multi-omics' and a powerful technology for the structural characterization of biomolecules and for analysis of proteins and small molecules such as lipids, sugars and metabolites. Combination of measurements from proteomics, genomics, epigenomics, transcriptomics and metabolomics, present a powerful decision-making format allowing deeper interpretation of a disease scenario in Precision medicine.

Expert commentary: Precision Medicine offers novel and promising ways to identify and treat a wide range of diseases. The future success of these therapies will be underpinned by novel proteo-genomic approaches linked to sophisticated databases to evaluate and predict drug-patient interactions.

ARTICLE HISTORY
Integra5 June 2020Received 11 April 2020
Accepted 3 June 2020Received 11 April 2020
Accepted 3 June 2020

KEYWORDS
Biomarkers; multi-proteomics; cancer; clinical proteomics; exosomes; mass Spectrometry Imaging; viral proteomics; precision medicine; systems Biology; socio-humanitarian

Since its success in Poznan, Poland in 2015, Central and Eastern European Proteomic Conference (CEEPC) was once again hosted in Poland but this time in Ustroń from September 23rd to 25th, 2019, with resounding success. CEEPC was founded to promote the 'power of proteomics' and has diligently propagated its Conferences with immense success. It was also the first to promote societal, humanitarian as well as global medical issues in parallel, both on its website and at Conferences, including Malaria, Ebola, Zika, and Coronaviruses. CEEPC has nurtured 'cutting-edge' research and multifaceted proteomics which is pivotal in everyday medicine including emerging viral challenges of today needing deeper understanding of viral proteomics. Proteins can undergo post-translational modifications, enzymatic cleavage and activation or destruction by proteolytic events. Such changes to proteins could be key to understanding disease onset or response to therapy. This is where proteomics plays a major role to decipher and characterize diseases. CEEPC's mission remains to contribute to all approaches of proteomics including '*novel enabling technologies*' to study diverse molecular processes or identify structural differences in proteins introduced by mutations, structural variations, modifications or protein truncation in disease. Multi-proteomics together with genomics and metabolomics stands to revolutionize global health.

When communism imploded around 1990, science in Poland suffered a dramatic financial collapse and an exodus of researchers. Joining the European Union in 2004 was very beneficial as it received funds to create new research facilities and to 'kick-start' scientific research. In this respect, Poland is the most successful transition country from communism in Central-Eastern Europe. As for its standing in proteomics, it seems en route to regaining lost strength and talent. Looking at the post-transformation in proteomic research in Poland and putting aside the legacy of the political system before 1989, it is embracing competitive international science, where Poland is becoming a force to be reckoned with.

CEEPC too has played a remarkable role in promoting proteomics in several of these similar Central and Eastern European countries [1]. Additionally, encouraging young researchers to present their findings orally or as posters and rotation of the meeting's venue each year to wonderful iconic locations of Europe, with diverse cultures, histories, fine cuisine, wines, and music, adds to the intertwining of 'cutting edge' proteomics and cultural excitement. To this day, CEEPCs' outstanding success is due to the undiminished enthusiasm of its unique group of friends, their academic institutional researchers, and participating scientists from all over the world, returning each year for this intoxicating mixture of proteomics and camaraderie [2–12].

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Piotr Widłak [13] and the organizing committee members – Franciszek Binczyk, Agata Kurczyk, Monika Pietrowska, Marta Gawin, Jacek Rogoliński & Karol Jelonek, put together a fascinating multidisciplinary program [14]. The theme of the Conference was 'Advances in Proteomics and Progress in Precision Medicine' and a whole plethora of proteomic disciplines were discussed including clinical proteomics, exosome proteomes, diverse 'multi-omics' topics including molecular imaging by Mass Spectrometry (MSI) and various 'novel enabling technologies.' The program also included dedicated time and a platform for young researchers' contributions via a 'Young Guns' session and a 'Posters session' with ample opportunity for networking.

Piotr Widłak (Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch, Gliwice, Poland) – chairman of the local organizing committee, inaugurated the Conference by warmly welcoming all participants. The thematic inauguration was elaborated by Suresh Jivan Gadher, Founder Member of CEEPC, highlighting the priorities of the CEEPC and charting the credibility, cohesion, vision together with reasons for its success [4]. Suresh also highlighted CEEPC's uniqueness in promoting socio-humanitarian issues [5] as well as proteomics related challenges including precision medicine, mental health, and epidemics impacting lives around the world where proteomics definitely has a role to play.

The keynote lecture was delivered by Theresa Whiteside (Department of Pathology, Immunology and Otolaryngology at the University of Pittsburgh School of Medicine, UPMC Hillman Cancer Center, Pittsburgh, USA) and was entitled, 'Tumor-derived exosomes as the emerging mechanism of cancer-induced immune suppression.' Theresa Whiteside is a world authority on 'Exosomes' in cancer and her presentation focused on tumor-derived exosomes (TEX). Exosomes were isolated from plasma, characterized and separated into subsets to study their molecular and genetic cargos to evaluate their contributions to reprogramming of the tumor microenvironment (TME), inhibition of host anti-tumor immunity and promotion of tumor progression. Interestingly, melanoma cell-derived exosomes (MTEX) isolated from plasma of melanoma emerged not only as surrogate markers of the tumor but also as biomarkers of the patients' immune competence.

The opening ceremony concluded with Czantoria Folk Ensemble which is famous in Eastern Europe for its repertoire based on the folklore of Cieszyn Silesia and festive songs [15]. This was followed by a traditional welcome dinner served with Polish hospitality which set the scene for the next day of the Conference.

Day 2 of the Conference commenced with the 'Biotechnology and New Approaches' session with a talk by Jacek R. Wisniewski (Max Planck Institute of Biochemistry, Martinsried, Germany) on 'Absolute quantitative proteomics of blood,' where mass spectrometry-based proteomics was compared to 'Total Protein Approach' (TPA) where the calculation of protein abundances is based on spectral intensities acquired on a large scale. Combination of the TPA method combined with Multi-Enzyme Digestion – Filter-Aided Sample Preparation (MED-FASP) together with sample digestion strategy – facilitated accurate quantification of plasma proteins and highlighted this technology as a powerful tool for investigation of blood cells and vesicles. A presentation on, 'The effect of low oxygen on the proteomic signature and functional features of pig Adipose Derived Stromal/Stem Cells (pASCs): Implication in skin wound healing' by Joanna Bukowska (Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland), employed diverse technologies of which Mass Spectrometry Imaging (MSI) analysis revealed that pASCs are sensitive to oxygen level alterations. Most of differentially expressed proteins reflected cell's adaptation to low oxygen tension, supporting pASCs survival and possible enhanced regenerative potential *in vivo*. A fascinating presentation entitled, 'Analysis of the antibacterial properties of *Naja ashei* snake venom components' by Aleksandra Bocian (Department of Biotechnology and Bioinformatics, Faculty of Chemistry, Rzeszow University of Technology, Rzeszow, Poland), focused on novel antibiotics from snake venom. *Naja ashei* venom is known to have antibacterial properties and studies showed that the phospholipase A2 and L-amino acid oxidases, as well as fragments of these enzymes, are mainly responsible for the bactericidal properties of venoms and possibly useful in designing of novel antibiotics effective against drug-resistant strains of bacteria. Similarly, Mariola Słowińska (Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland) added to the excitement by presenting her research on the cysteine-rich protein identified as cysteine-rich venom protein-like isoform X2 (CRISP-V) which is most abundant in turkey seminal plasma and yet its functionalities are not fully understood. This session concluded with a presentation from Robert Nawrot (Adam Mickiewicz University, Institute of Experimental Biology, Poznan, Poland) who discussed Major latex protein (CmMLP) isolated from medicinal plant *Chelidonium majus*. Using enabling technologies, this study highlighted the presence of alkaloids and low molecular compounds, interactivities of which may be responsible for the beneficial properties including antimicrobial, antiviral, antitumor, anti-inflammatory, antifungal, and fungistatic properties, which could be utilized for benefit of mankind.

The Clinical Proteomics session commenced with a presentation from Karoly Vékey (Hungarian Academy of Sciences, Budapest, Hungary) who presented a talk on, 'Changes of the IgG (Immunoglobulin G) glycosylation in Rheumatoid Arthritis (RA)' using LC-MS/MS (liquid chromatography (LC)-linked tandem mass spectrometry) and applying both low and high energy CID (Collision-induced dissociation). Low energy CID studies were useful for evaluating complex sample mixtures. Glycopeptide analysis showed that glycosylation among RA patients was greater compared to control patients. Similarly, a presentation on 'Proteomic analysis of the human follicular fluid from women undergoing *in vitro* fertilization (IVF)' from Aleksandra E. Lewandowska (University of Gdańsk, Gdańsk, Poland), employed FASP (Filter Aided Sample Preparation) technique and SWATH (Sequential Window Acquisition of All Theoretical Fragment Ion Spectra) mass spectrometry. Such technologies can facilitate the identification of protein biomarkers in IVF as well as potential protein markers of oocyte quality in follicular fluid.

An informative presentation from Piotr Widlak entitled, 'Molecular heterogeneity of papillary thyroid cancer: comparison of primary tumors and lymph node metastases by MALDI-MSI' (Matrix-assisted laser desorption/ionization mass spectrometry

imaging). Piotr utilized various technologies including MALDI-TOF MSI (matrix-assisted laser desorption ionization (MALDI)-time of flight (TOF) mass spectrometry) for tryptic peptides together with LC-MS/MS (liquid chromatography (LC)-linked tandem mass spectrometry) approaches for tumors and was able to show a marked molecular difference between primary thyroid cancer and its lymph node metastases, concluding that phenotypical intra-tumor heterogeneity between primary tumor and lymph node metastases from the same patient was higher than inter-tumor heterogeneity between primary tumors from different patients.

The importance of Biomarkers was once again highlighted by a presentation on Vulvar Squamous cell carcinoma (VSCC) by Magdalena Kowalewska (Department of Molecular and Translational Oncology, Maria Sklodowska-Curie Institute – Oncology Center, Warsaw). Often, topics related to female genitalia are approached with embarrassment and reticence, however, Magdalena tackled the seriousness of her research forthright by showing, 'The Great Wall of Vagina' by Jamie McCartney [16], a piece of art created to address social stigmas and misconceptions. Tumor and normal valvar tissue were studied using iTRAQ (Isobaric tags for relative and absolute quantitation) analysis. Correlation of the results with clinical parameters of the enrolled patients indicated that High Mobility Group AT-Hook 2 (HMGA2) and Proteinase 3 (PRTN3) as potential protein markers for the prediction of VSCC progression. Increased HMGA2 and PRTN3 tissue abundance and higher PRTN3 blood levels were associated with the aggressive phenotype of VSCC.

The Clinical Proteomics session was concluded by Monika Pietrowska (Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch, Gliwice, Poland) with a presentation on the Proteomes of exosomes from HPV(+) or HPV(-) head and neck cancer cells: differential enrichment in immunoregulatory proteins. Human papillomavirus (HPV) is an etiologic factor in head and neck squamous cell carcinoma (HNSCC). HPV(+) cancers respond favorably to therapy potentially due to more robust anti-tumor immune responses. Monika hypothesized that tumor-derived exosomes (TEX) produced by HPV(+) or HPV(-) HNSCCs differentially modulate anti-tumor immune responses. Studies using high-resolution mass spectrometry and biological activity of selected immunoregulatory proteins were validated by flow cytometry and co-incubation assays. This research concluded that the differential content of protein cargos in HPV(+) and HPV(-) exosomes might contribute to the disparity in immune responses that characterize HPV(+) and HPV(-) HNSCC.

The final 'Metabolomics session' of the day commenced with a presentation by Gwendolyn Barceló-Coblijn (Palma, Spain) on, 'MALDI-IMS lipidomic data to study cell signaling pathways: the importance of the lateral resolution.' Using MALDI-IMS to analyze human colon mucosa sections, Gwendolyn demonstrated that not only the lipidome is cell-type specific but also that it is highly sensitive to any change in the pathophysiological state of the cell. These analyses revealed how precisely a very specific set of lipids change along the colon crypt. Altogether, results indicated a complex interaction between membrane lipids and prostaglandin metabolism in colonocyte differentiation and tumorigenesis.

Karol Jelonek (Maria Sklodowska-Curie Institute – Oncology Center, Gliwice) presented, 'Systemic effects of radiotherapy and concomitant chemo-radiotherapy in head and neck cancer patients – comparison of serum metabolome profiles.' Karol's research compared profiles of metabolite in the serum of head and neck cancer patients treated with concomitant chemo-radiotherapy (CCRT), radiotherapy alone (RT) or chemotherapy alone (ICT; induction treatment during sequential chemo-radiotherapy). Concomitant chemoradiotherapy induced the quickest and the most severe systematic changes observed in serum metabolome, which affected mostly its phospholipid component. Understanding how human metabolism is influenced by mentioned treatments is crucial to predicting individual response and for adjustment of personalized therapies in Precision medicine.

Łukasz Marczak (Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland) concluded the day's proceedings with a presentation on functional analysis in plants using sophisticated metabolomic approaches for plant metabolite screening. Łukasz reiterated the importance of metabolomics and the importance of plant proteomics in Precision medicine.

The majestic sight of *Dwór Skibówki* [17], a gastronomic restaurant situated on top of Równica mountain and offering panoramic views of distant Tatra Mountains, was the venue for the evening dinner offering traditional food, drinks, and folklore entertainment. Once again, CEEPC's multiculturalism was not only evident but also imbibed and enjoyed by all late into the night.

The final day of the Conference was a mixture of intense as well as relaxed sessions and encompassed the Poster session, the 'Young Guns' session as well as the 'New Concepts and Methods' and the 'Proteo-genomics and Molecular Networks' sessions. Morning commenced with the 'Young Guns' session which included numerous short presentations of the highest caliber from young researchers. CEEPC provides a unique platform to young researchers for presenting their latest research projects, novel ideas, optimism, and their excitement. Often such sessions push the boundaries of medical and scientific research, pointing to new directions and dimensions with immense research benefits. To this end, CEEPC remains indebted to its young researchers for their incredible hard work and enthusiasm which also makes CEEPC what it is today!

The 'New Concepts and Methods' session was added to the program to highlight the advances in the *'enabling technologies'* which continue to facilitate advances in Precision Medicine. This session commenced with Joanna Polańska (the Silesian University of Technology, Data Mining Division, Gliwice, Poland) who presented, 'Machine Learning in the processing of proteomics data.' Mass Spectrometry Imaging (MSI) is a powerful tool in Proteomics and enables untargeted investigations into the spatial distribution of molecular species in a variety of biological specimens but also brings a tremendous amount of data that require dedicated algorithms for signal analysis. Joanna has developed the comprehensive MSI data processing pipeline consisting of the Gaussian Mixture Model-based feature extraction, 'intelligent stepwise divisive k-means clustering algorithm' for tissue heterogeneity modeling and Monte Carlo procedure (MI) for the identification of the most critical features and interactions

between features distinguishing healthy tissue from the tumor region. Joanna demonstrated the system performance on the MSI proteomic data collected from the head and neck, thyroid and prostate cancer tissue samples.

Laszlo Drahos (RCNS of Hungarian Academy of Sciences, Budapest, Hungary), presented on the effect of post-translational modifications (PTMs) on the number of identified proteins using Byonic *(software search engine for peptide and protein identification by tandem mass spectrometry)* and MASCOT *(software search engine to identify proteins from peptide sequence databases)*. Several hundreds of searches were performed to discover general trends and determine when it was worth considering a PTM in a protein search. This presentation showed that a Byonic search engine can handle a lot of modifications and one of its real advantages was to keep search space under control. Results showed that as a rule of thumb it is worth considering a PTM if its occurrence is more than 2.5% in case of Byonic searches.

Marta Gawin (Maria Sklodowska-Curie Institute – Oncology Center Gliwice Branch, Gliwice, Poland), shared her expertise on the application of mass spectrometry imaging (MSI) techniques for the analysis of archived tissue specimens in the form of formalin-fixed paraffin-embedded (FFPE) material which has opened up broad possibilities in research on various diseases, including cancer. Marta cautioned the participants about the treatment of the resected material and the resulting chemical modification of the tissue which can impose certain challenges, and which need to be addressed at every stage of sample preparation including heat-induced antigen retrieval process for MSI.

The concluding session on 'Proteo-genomics and Molecular Networks' commenced with Maciej Lalowski (Medicum, Biochemistry/Developmental Biology, Meilahti Clinical Proteomics Core Facility, University of Helsinki – Helsinki, Finland) entitled, 'Dissecting rare disorders: from compartmental proteomics towards affected pathways and identification of putative drugs.' Maciej discussed Neuronal ceroid lipofuscinoses (NCL) which are progressive encephalopathies of childhood with severe consequences. Of these, NCL5 is a rare late-infantile form of neuronal ceroid lipofuscinosis where the primary function of CLN5 protein and its physiological roles remain unresolved. Emerging evidence pointed to mitochondrial dysfunction in the onset and progression of several NCL forms, offering new insights into putative biomarkers and shared biological processes. Suresh Jivan Gadher (Thermo Fisher Scientific, Carlsbad, CA, USA) presented a holistic investigation of a single biological sample. Often measurement of mRNA expression or protein levels alone may not tell a complete story as mRNA expression levels may not translate to protein. Combining messenger RNA (mRNA) expression using QuantiGene™ Plex assay with protein detection using ProcartaPlex™ immunoassay, an amenable solution for high-level interrogation of both genomic and proteomic parameters is achieved from the same patient sample. Such 'proteo-genomic' approach is helping to screen a large number of samples for the molecular signature of human diseases at both protein and gene level in Precision medicine.

Marek Rusin (Maria Skłodowska-Curie Institute – Oncology Center, Gliwice Branch, Gliwice, Poland) shared his belief that strong activation of p53 tumor suppressor protein is associated with coordinated upregulation of proteins protecting against infections. Marek investigated if an array of genes coding for proteins of the first line of defense against various viruses and bacteria is regulated by p53 protein by exposing various cancer cell lines to camptothecin or actinomycin D with nutlin-3a. The genes regulated in p53-dependent fashion were identified using model cell lines with p53 expression *knock-down* by shRNAs or *knocked-out* using CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9) technology and expression of selected genes at protein level were examined using immunodetection methods. Marek concluded that p53 had the ability to fight infections caused by various pathogens including bacteria and viruses as judged by the functions of other proteins induced by strong activation of p53.

Jadwiga Jablonska (Translational Oncology, Department of Otorhinolaryngology, University Hospital Essen, 45,147 Essen, Germany) concluded the final session of the Conference with a presentation entitled, 'Identifying head and neck cancer (HNC) biomarkers using proteomic analysis of neutrophils and neutrophil-derived exosomes. Jadwiga set out to explore if extracellular vesicles, mainly exosomes, released from neutrophils might play a critical role in neutrophil-dependent regulation of tumor development and spread via mediating communication between tumor-associated neutrophils and target cells present in tumor or metastatic niche. This research showed that exosomes are involved in tumor development and that metastasis and exosome protein contents may harbor key biomarkers useful in identifying HNC in patients.

The conference concluded with closing remarks by the organizers. CEEPC had excelled in facilitating expert scientific interaction and collaborations between research specialties in Systems Biology and Proteomics, propagating interest in protein functionalities in health and disease. Additionally, the 'Young Guns' session provided a new momentum and a fresh outlook to the young generation of researchers fascinated by proteomics related Precision medicine. The conference was a great success and many participants stayed behind to enjoy not only the recreational activities of the Spa town of Ustroń but also the health benefits of the mineral-rich curative springs and layers of therapeutic mud at its resorts.

A well-earned 'farewell conference dinner' with all its Polish hospitality and traditional cuisine, music and dancing marked the end of a very successful Conference. Unlike other conferences where participants disperse in a hurry at the close, this relaxed atmosphere allowed for further networking. Proteomics remains dominant in scientific, medical, and even more so now in clinical research due to the Coronavirus disease 2019 (COVID-19). Proteomics is increasingly followed by diverse research groups all over the world. As these research groups and their areas of interests expand and excel, proteomic community such as the Central and Eastern European Proteomic Conference and the European Proteomics Association (EuPA) provide a forum to pursue agendas that meet their key objectives.

CEEP conference is held every year in a different location in Central or Eastern Europe. However, this year in view of the rapid developments pertaining to the COVID-19 pandemic and travel restrictions, we have decided to cancel the 14th

CEEPC, 2020 that was to be held in Budapest, Hungary from 14 to 16 October 2020 [18]. We apologize for any inconvenience and we look forward to year 2021 when we can meet up again at a fitting location to once again enjoy proteomics as well as discuss the pressing societal challenges facing mankind.

Expert Opinion

Precision medicine is an emerging medical concept even though it has been around for a long time but with limited examples in reality. The goal of Precision Medicine remains to find general treatments that are highly effective for large numbers of individuals who fall into precisely diagnosed groups. Diseases develop over time in defined biological steps, and these steps may differ among individuals based on genetics and environmental conditions. Utilizing proteogenomics and big data together with patient engagement, is helping individualize medical care, develop rational therapies and preventive measures based on precise understanding of the steps leading to the clinical expression of diseases.

At this important juncture with advances in proteomics and evolution of '*novel enabling technologies*', the study of the human proteome, metabolome and genome has a vital role to play. Researcher and clinicians see this as an opportunity to advance beyond generic research to better understand disease treatments and prevention by considering an individual's overall phenotype which is greatly influenced by demographic characteristics, variability in genes, environment, lifestyle and epigenetics. Given that the individual's phenotype is dynamic and ever changing due to multiple factors including stress, mental health, aging, microbiome and more recently enhanced environmental challenges, proteomics provides the power to interrogate the proteome which has the insight into physiological and biological parameters which can be measured from cells, tissues or organs.

Combination of multiple quantitative measurements from proteomics, genomics, epigenomics, transcriptomics, metabolomics and microbiome, present a powerful decision-making format allowing deeper interpretation of a disease scenario in Precision medicine. With the possibility of large data collection, comes the challenges of defining data types that are valuable for specific purposes such as disease prediction, drug efficacy or biomarker identification. Since 'Big data' generated is not 'Smart data', the pressing challenge remains to convert this data and findings into predictive quantitative models of on-going cellular processes where information from proteomics, 'multi-omics' and clinical phenotype is needed to realize the end goal of Precision medicine. Through 'Machine learning' and artificial intelligence (AI), we can reason about and utilize data at an unprecedented scale in order to *predict, prevent, and treat disease more effectively*. Moving forward, 'Machine learning' and AI will eventually close the loop to harness the predictive power of an individual's data to ensure that one does not receive the wrong medication – *we are now talking about the 'futuristic pharmacy' within Precision Medicine!*

In summary, power of proteomic and continued progress in Precision Medicine will hopefully enable us to classify a disease more precisely, treat patients on individual indication at that specific stage of the disease with better success as well as put into place preventative measures for the benefit of mankind.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Funding

This paper was not funded.

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