Proteomics
www.proteomics-journal.com

Clinical Proteomics: Closing the Gap from Discovery to Implementation

*Maria Frantzi, Agnieszka Latosinska, Georgia Kontostathi, and Harald Mischak**

Clinical proteomics, the application of proteome analysis to serve a clinical purpose, represents a major field in the area of proteome research. Over 1000 manuscripts on this topic are published each year, with numbers continuously increasing. However, the anticipated outcome, the transformation of the reported findings into improvements in patient management, is not immediately evident. In this article, the value and validity of selected clinical proteomics findings are investigated, and it is assessed how far implementation has progressed. A main conclusion from this assessment is that to achieve implementation, well-powered clinical studies are required in the appropriate population, addressing a specific clinical need and with a clear context-of-use. Efforts toward implementation, to be feasible, must be supported by the key players in science: publishers and funders. The authors propose a change on objectives, from additional discovery studies toward studies aiming at validation of the plethora of potential biomarkers that have been described, to demonstrate practical value of clinical proteomics. All elements required, potential biomarkers, technologies, and bio-banked samples are available (based on today's literature), hence a change in focus from discovery toward validation and application is not only urgently necessary, but also possible based on resources available today.

Proteins represent the key building blocks of every organism. If the exact functional and structural information and networking capabilities on all proteins in an organism was available, then it should be possible to exactly define all functions of this organism, its health status, and disease at the molecular level. Such or similar considerations are a cornerstone of proteomics, which aims at assessing the proteome in a holistic manner, ideally all proteins contained in the sample under investigation.[1] Diseases are generally the result of proteomic changes with the (molecular) pathology being a consequence of specific changes in protein structure, abundance, and/or function. Not surprisingly, in general, all drugs act at the proteome level, more or less specifically targeting proteins.[2] As such, the field of clinical proteomics was developed, aiming at deciphering the changes in proteins that are relevant in disease, thereby providing ideal biomarkers for diagnosis, prognosis, and prediction of therapeutic response, and supporting drug development.

Mainly based on its enormous hypothetical relevance, clinical proteomics is an ever-growing field. When investigating the recent literature in web of science (using the search terms **clinic*** AND **proteom*** OR **proteom*** AND **biomarker**), approximately 1000 manuscripts are published yearly, with numbers increasing every year (**Figure 1**).

In principle, two major areas of clinical proteomics applications are apparent, with different goals and requirements. This has a significant impact on the study design, as depicted in **Figure 2**. The first and more widely addressed application is the identification of biomarkers for improvement in diagnosis, prognosis, or prediction (of therapeutic response).[3] This approach requires the specimen to be readily available and a large number of samples (typically exceeding 1000), to ascertain the performance of the potential biomarker. Moreover, validation studies in appropriately selected independent cohorts are necessary to ensure successful implementation. The other major application is the identification of targets for intervention or of therapeutic drugs.[4,5] In this case, the ideal specimen is the affected tissue, to inform about molecular changes in disease (**Figure 3**). Due to also the limited availability of tissue, the number of samples to be investigated is typically lower than in biomarker studies. A widely applied approach includes the identification of a large number of significant changes that in combination with already published data, enable defining molecular pathology and pathways affected in a comprehensive way. In several recent articles, this approach is presented in detail; as an example, we refer to a review in the context of chronic kidney disease (CKD).[6] Such data should enable the identification of potential key molecules in the pathological processes that could serve as rationale drug targets.

The identification of drug targets or candidates obviously is more demanding, consequently most published studies focus on the identification of potential biomarkers. Deviations from the general scheme exist (e.g., a proteomic change in the tissue may

Dr. M. Frantzi, Dr. A. Latosinska, Dr. H. Mischak
Mosaiques Diagnostics GmbH
Hannover, 30659, Germany
E-mail: mischak@mosaiques-diagnostics.com
Dr. G. Kontostathi
Department of Biotechnology
Biomedical Research Foundation Academy of Athens
Athens, 11527, Greece

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/pmic.201700463

DOI: 10.1002/pmic.201700463

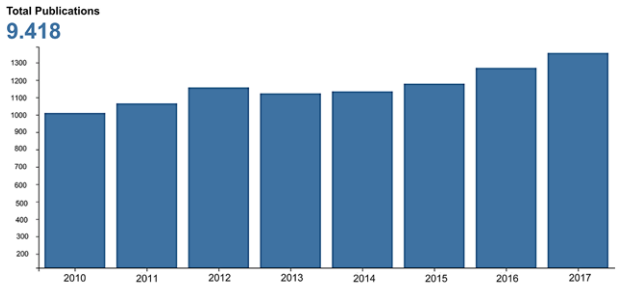

Figure 1. Number of manuscripts published and citations received from 2010 to 2018 when searching the Web of Science for the topics "clinic* AND proteom*" OR "proteom* AND biomarker", excluding reviews and meeting abstracts. It is evident that the number of studies is increasing each year, indicating a growing field.

also be further investigated for its potential role as biomarker in body fluids,[7] biomarkers in body fluids may be linked to disease pathophysiology and support definition of potential drugs,[8] etc.), but overall clinical proteomics approaches generally fit in one of these schemes.

When investigating for the outcome of the huge efforts in clinical proteomics (as evident from the thousands of papers published), multiple biomarkers and drugs are expected to be applied in patient care. However, the results do not fully meet these expectations. We were not able to identify a drug at least being tested in a clinical trial that was developed as a result of a proteomics driven study. This may to a degree be attributed to the enormous challenges associated with developing a drug candidate and the regulatory hurdles that accompany clinical trials.

However, and giving rise to a more optimistic view, several candidates, drugs, or drug targets that were derived from a proteomics study showed benefit in relevant model systems. Ren et al. performed comparative proteome analysis of the human hepatoma cell line (HepG2) and an immortal hepatic cell line (L02) and identified phosphoglycerate mutase 1 (PGAM1) as potential candidate.[9] Knock-down of PGAM1 inhibited cancer cell growth, providing evidence for the potential of this protein as a drug target in hepatocellular carcinoma. Bone marrow stromal antigen 2 (BST2) was found increased in endometrial cancer using proteomics[10] and a therapeutic potential of a monoclonal antibody against BST2 was demonstrated. When investigating cellular models of insulin resistance (3T3-L1 adipocytes treated with TNF-α or dexamethasone) with the aim to discover potential therapeutic targets for obesity,[11] progranulin (PGRN) was identified as a differentially regulated protein. Ablation of this protein (Grn(-/-)) prevented mice from insulin resistance

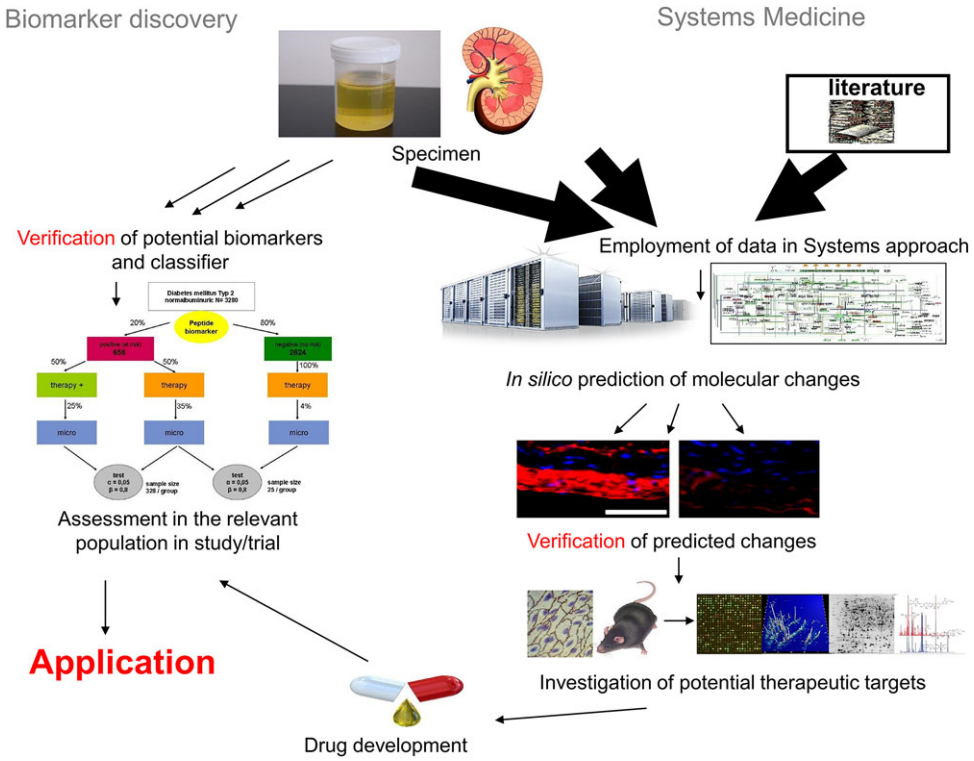

Figure 2. Graphical depiction of the main two different routes that clinical proteomics approaches can take: toward biomarker discovery, or a drug development based on systems medicine approach. The biomarker approach (left) requires identification of distinct potential biomarkers that are subsequently verified, and then applied in the appropriate clinical setting. If benefit can be demonstrated, they should be implemented/routinely applied. The drug development approach (right) takes advantage of the breadth of data, including literature sources, aims at molecular modeling of disease and predicting key structures. These are then verified (first valid result), and subsequently their value as potential targets is investigated in appropriate interference studies. If positive, drugs can be developed, tested, and, if beneficial, should be applied. Starting with the same or similar data, the downstream utilization (and the associated issues) is quite different, but ultimately in both cases aimed at investigating proteomics to improve patient care.

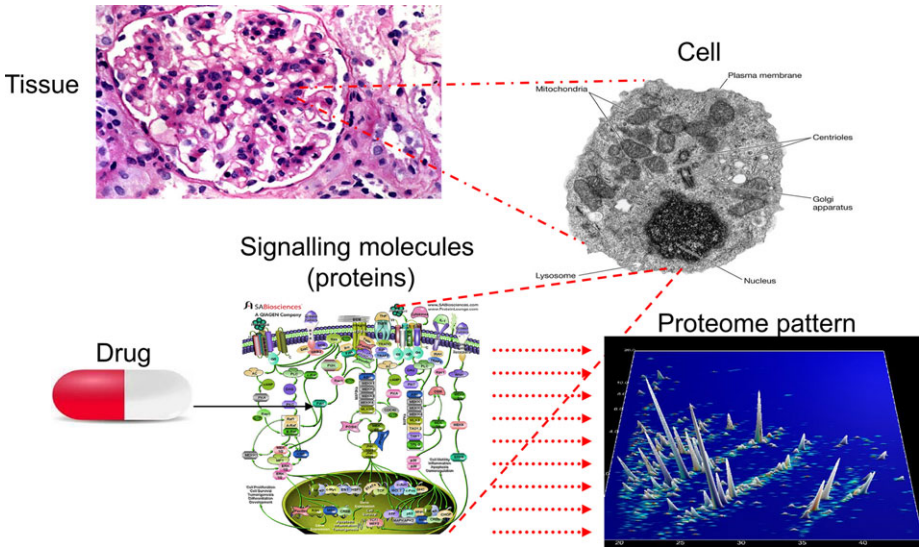

Figure 3. Changes in the proteome display molecular pathophysiology. Microscopic investigation of tissue can reveal structural changes. However, subcellular structures, and even more the molecules involved in the molecular pathology, can generally not be assessed. In contrast, proteome analysis does not give information on morphological changes, but gives information on global protein changes which can be associated with the molecular changes in disease. Some of these molecules represent the most appropriate targets of therapeutic drugs; hence, proteome analysis can give guidance on the molecular structures to be targeted in therapy. Reproduced with permission.[2] Copyright 2015, the author; published by Oxford University Press.

induced by high fat diet and obesity, mediated though the blockage of IL-6 production. Proteome analysis of tissue from non-muscle-invasive (NMIBC) and muscle-invasive bladder cancer (MIBC) followed by pathway and interactome analysis revealed a functional significance of eukaryotic initiation factor 3D (EIF3D), overexpressed in MIBC.[12] Silencing of EIF3D decreased tumor cell proliferation, colony formation, and tumor growth in xenograft models.

The outcome is slightly more positive with respect to biomarkers: a few proteomics-based biomarkers made it to clinical application, at least in the context of appropriate clinical trials (reviewed below). Proteins are certainly frequently employed as biomarkers on a routine basis to assess a variety of pathologies,[13] further underlining their relevance and value in this context. However, these protein biomarkers that are routinely applied were generally not discovered as a result of a proteomics screen. The data currently available also indicate that single biomarkers in general do not allow detecting disease or progression with high accuracy. This has resulted in the application of multi-marker panels that have shown superiority, significantly higher accuracy when compared to single biomarkers.[14,15]

1. The First Success Stories

When examining clinical trials.gov for the keywords: "**proteome**," "**proteomics**," "**peptidome**," or "**peptidomics**", and filtering for interventional studies, 334 entries are returned. Following manual inspection, only two of these studies were found to aim at actual application of proteome analysis in patient management. All other studies are observational without implementing proteomics in guiding clinical decision-making, and/or without giving specific information. NCT02040441 (Proteomic Prediction and Renin Angiotensin Aldosterone System Inhibition Prevention of Early Diabetic Nephropathy in Type 2 Diabetic Patients with Normoalbuminuria, PRIORITY), applies the previously developed mass spectrometry–based classifier CKD273[16–19] in diabetic patients that do not have any sign of kidney disease. Patients scoring positive in CKD273 (predicted to develop clinically evident CKD in the near future) are randomized for treatment with spironolactone or placebo.[20] The aim of the trial is to 1) demonstrate validity of CKD273 in the early detection of diabetic nephropathy, and 2) demonstrate that early intervention with spironolactone will reduce CKD, assessed via the surrogate parameters albuminuria and eGFR. NCT03116217, Validation of a Fetal Urine Peptidome-Based Classifier to Predict Post-natal Renal Function in Posterior Urethral Valves (Antenatal), evaluated a mass spectrometry–based classifier of 12 peptides in fetal urine that predicts end stage renal disease after birth[21] in fetuses with bilateral congenital abnormalities of the kidney and urinary tract (CAKUT). Bilateral CAKUT displays a wide spectrum of pre and postnatal outcomes ranging from death in utero to normal postnatal renal function. Methods to predict these outcomes in utero are controversial and, in several cases, lead to unjustified termination of pregnancy. Peptides discovered in fetal urine highly significantly improve prediction, and employment of appropriate intervention strategies.

When examining the ISRCTN registry, 16 trials are returned, one of them aiming at the application of proteome analysis in patient management, ISRCTN03911524: Pre-emptive Therapy of Acute Graft Versus Host Disease (GvHD) According to Specific Proteomic Patterns after Allogeneic Haematopoietic Stem Cell Transplantation. In this multicenter randomized clinical trial (RCT) a validated proteomics-based multi-marker assay is used for the prediction/early detection of GvHD.[22] Patients scoring positive for GvHD are randomized for treatment with

corticosteroid or placebo. The aim of the trial is to demonstrate an improvement in the prediction of GvHD, and a benefit of the early/pre-emptive intervention with corticosteroid.

Obviously, the expectation that we should be in a position to have multiple proteomics biomarkers and drug targets at our disposal is not really met by reality. To find an answer to the question why this huge discordance between expectation and reality is observed, the currently published research results on this topic were assessed.

2. The Typical Result

When investigating a random sample of manuscripts retrieved using the search strategy employed for Figure 1, only 10–20% of the manuscripts report on an actual potentially clinically relevant application, the others are to a large degree technical papers on, for example, sample preparation, on different proteomics techniques, and to a minor extend manuscripts on animal specimens. To a substantial extent reviews, viewpoints, and manuscripts not truly relevant to the topic, for example, studies on miRNA, are also present.

When examining a random sample of the published clinical proteomics studies, many of them present similar drawback(s): The vast majority of papers describe exploratory studies, resulting in "potential biomarkers" that should be tested by somebody else. Even in studies that include a cohort where the potential biomarker was initially verified, the following issues are frequently observed:

a) studies are underpowered for the specific context-of-use, frequently presenting low sample sizes
b) the new biomarkers are not tested for their potential of significantly improving the current methods and/or state of the art (implemented biomarkers or other diagnostic tools)
c) the study is not performed in the population of interest: for example, when proposing a screening test for a condition with prevalence of 1/1000, this must be tested in a cohort with similar distribution. Developing and testing the potential biomarkers in a cohort where the distribution is 50/50 is inappropriate, resulting in unrealistic claims
d) the biomarkers are not tested for a specific meaningful context of use and claims by far exceed the results. For instance, a biomarker in a case control study comparing healthy subjects with patients with advanced tumor may well be able to distinguish between these two conditions with 85% accuracy, but this does in no way indicate that the biomarker could enable early detection of the tumor with 85% accuracy (sensitivity and specificity). In addition, based on a prevalence of 1/1000 (which is not unrealistic), this still would result in about 177 false positives for a single correctly identified case, a result which is completely ineffectual for screening purposes.

3. Technical Considerations

Most proteomics discovery studies involve LC-MS/MS, as it enables high resolution analysis of specimens with increased complexity and allows for a comprehensive protein identification and quantification, by enabling assessment of thousands of tryptic peptides.[23] To complement with the detection of different isoforms and post-translational modifications, mass spectrometry can be combined with 2D electrophoresis as a prior separation step.[24] Clearly, when it comes to validation studies, the biomarkers need to be further investigated in a high throughput and reproducible manner. The obvious limitations of the above technologies in terms of throughput can be overcome with targeted proteomic approaches like multiple, selected, and parallel reaction monitoring (MRM, SRM, and PRM). Targeted protein quantitation by the above technologies is currently applied for validation studies, as an alternative to immuno-based single (ELISA) and multiplex immuno-assays, as they are generally more selective, suffer less from matrix effects and also synthetic peptides can be readily available for all targeted biomarkers.[25]

For the discovery and validation of low molecular proteome (peptidomic) biomarkers, capillary electrophoresis coupled with mass spectrometry (CE-MS) has been established as a robust method with numerous clinical applications to date.[26,27] This technique involves separation of the small proteins and peptides in narrow-bore fused silica capillaries based on their electrophoretic mobility, and on-line detection using MS. Analytical validation in terms of reproducibility, repeatability, and stability of the platform in clinical conditions has been demonstrated, and appropriate standard operating protocols are established.[28]

Evidently, technical issues do not generally stand in the way of implementation of proteomic biomarkers. It is undisputed that additional improvements in technology may be beneficial and further ease the implementation of proteomic biomarkers, but it must be acknowledged that the technologies have advanced sufficiently to allow routine application in clinical diagnosis or prognosis, as evident from the plethora of manuscripts on technologies to be applied for clinical proteomics. This is especially pronounced for targeted mass spectrometry like MRM and similar approaches, which have been developed and advocated for years and which present a solid and well-established technology, ready for implementation.[25,29,30]

Similar considerations apply for the (bio)informatic analysis. Improvements in the (bio)informatic assessment of the raw data certainly have the potential to add to the current efforts, improving the available pipelines for biomarker research. However, these efforts do not appear to be mandatory prior to implementation. The current (bio)informatic solutions are stable, have demonstrated robustness and are ready to be used for the routine evaluation of data. While the benefit of further development is not disputed, such efforts should not stand in the way of implementation.

4. Obstacles and Hurdles: An Attempt to Learn from the Past

Overall, multiple clinical proteomics studies are published reporting valid results that can be reproduced. At the same time, it appears the results are over interpreted even in well-performed studies. When considering a biomarker as a feature that can be measured with confidence that gives specific information on a specific pathology, for a specific and useful context-of-use, and adding the criterion that any new biomarker must demonstrate

a significant improvement/addition over the current state of the art, then most studies published do not fit these strict, but very reasonable and useful criteria. The very liberal use of the term "biomarker" is not helpful. The mere potential association of a feature with a pathology or a specific condition (which is what in general is published) does not constitute sufficient evidence to label the protein a biomarker.

An important criterion is the exact definition of the context-of-use for a biomarker. This (context-of-use) should be linked to a therapeutic/interventional consequence following the biomarker readout which further necessitates specific biomarker cutoffs to be determined (a value above the cutoff results in a specific action/intervention). Although this issue has been strongly advocated in the past, unfortunately moderate attention is paid to it. When examining the 100 most recent manuscripts, based on the condition to include the word "**biomarker**" in the title, and **proteom*** and **clinic*** in the topic, then in only a very small fraction of the papers returned a well-defined context-of-use is presented as a basis of the study. Although the definition of a well-defined context does not appear too relevant, especially not for a proteomics researcher, a biomarker is defined by its context, hence the definition is mandatory.

Similar considerations apply for the claim of a "potential biomarker," which typically refers to a protein that apparently differs in distribution in the samples investigated, but for which no substantial evidence for any real value in a specific context has been presented. Frequently, appropriate statistical analysis is not performed, adjustment for multiple testing is avoided. As a result, the "potential biomarkers" with very high certainty represent artifacts. The result of avoiding multiple testing and performing underpowered studies has very impressively been demonstrated by Danka et al.[31] The authors could show that essentially all "potential biomarkers" resulting from such a study cannot be verified in a validation cohort and are not associated at all with the condition examined. The consequences of avoiding multiple testing can be easily evaluated by every reader: if randomly choosing 14 proteomics datasets (containing information on >1000 proteins) from a control population and randomly splitting the data in seven cases and seven controls, then the application of a simple statistical test will return multiple apparently significant differences between the groups, for a target p-value <0.05 slightly above 5% of the number of proteins in the input file. If the original set of 14 is in a second experiment split in another random samples of seven cases and seven controls, then the same statistical test will give about the same number of apparent significant differences, but the overlap between the two experiments will be close to 0. This impressively demonstrates the mandatory requirement for adjustment for multiple testing.

The approach to present underpowered studies in combination with "relaxed" statistical analysis and unjustified claims is based on the (unfortunately correct) assumption that a "potential biomarker," even if no potential has been demonstrated, can nevertheless be published. Considering the fact that scientists are evaluated based on their publications, it certainly is much more cost-effective to publish a "finding" based on highly preliminary data (and just add a couple of more or less unjustified claims to ensure acceptance), rather than performing a well-planned study, that requires substantially more time and effort, and holds the risk of being rejected as "not novel." However, such approaches unfortunately at the same time also have a substantial negative impact on the field of clinical biomarkers: reliability of reported biomarkers is questioned especially by clinicians, as a result of biomarkers generally being reported in the absence of the evidence required.

Surprisingly, the obvious need for implementation studies does not appear to fully meet with the priorities of publishers and funders. When examining the recently published literature, the majority of published studies are either technical reports, or reports on "potential biomarkers," on exploratory discovery studies in by far too small cohorts. Along these lines, the application or thorough testing of the value and validity of a biomarker is frequently considered neither publishable nor fundable, with arguments among others that such a study does not represent a scientific discovery, is not novel, should be left to industry, etc. There are certainly exceptions to this observation, but these represent the minority.

In the past and in multiple manuscripts, a change toward performing well-powered studies that would enable validation and qualification of biomarkers has been proposed.[3,32–34] However, if such studies do not meet the necessary support of the scientific journals and the funders, it is foreseeable that they will generally not be undertaken.

Even if clinical proteomics studies are performed properly, demonstrated a significant benefit, and even indicate cost efficiency, implementation is not straight forward. Major obstacles and hurdles have been detailed by, for example, Mischak et al.,[33] and are highlighted in a recent article.[35] As expected, major challenges are to convince regulators and payers. A useful approach for how to cope with these obstacles is approaching these organizations as soon as possible, ideally even prior to initiating the studies. This is especially true when it comes to regulators. Specifically the US-FDA has in the past been helpful when aiming at developing biomarkers, and discussions with this agency may ultimately lead to the recognition of a biomarker, which can be beneficial in implementation.[36] To date, the experience with payers is less positive: it becomes now evident that, for example, the G-BA, the authority deciding on reimbursement by the public health insurance in Germany, lacks the required knowledge and competence at least in clinical proteomics and biomarkers.[35]

5. The Consequence: Suggestions How to Proceed from Here

5.1. Biomarker Research

Based on the evidence available, multiple well-developed technologies are available for clinical proteomics. In addition, a plethora of reports on biomarkers (with more or less actual value and validity) is available. It appears that the next logical step is the application of potential biomarkers (apparently best of those that are supported by decent evidence and appear credible) in well-powered studies, in the population representing the biomarker context-of-use, and taking into account the current state-of-the art. The aim of such a study would be the demonstration of a significant benefit over or in addition to the current state-of-the-art (e.g., demonstrating a significant improvement in the prognosis of progression in CKD over albuminuria, the currently used

method).[19,37,38] We want to emphasize the importance of subsequently publishing such well-performed studies, even if they are negative. Along with power calculations, an indication for a health economic benefit should be presented, as a biomarker likely will only be implemented if a health economic benefit can be shown.

A candidate for such a study is HF1; a classifier based on 85 urinary peptides that was established to discriminate individuals with subclinical left ventricular diastolic dysfunction and controls with good performance (AUC of 0.84).[39] Additional studies have indicated a benefit of HF1 in the management of left ventricular diastolic dysfunction,[40] including the ability to predict incident cardiovascular and cardiac disease over a 5 year period.[41] Based on the evidence presented in these large studies, a properly powered trial to demonstrate its clinical value and subsequent implementation now seems the logical path forward.

To ease the progress toward the major goal, the implementation of clinical proteomics in patient management, we propose a five-step process:

1) define the clinical need, together with the anticipated consequences of the implementation of the biomarker(s), assuming they present an improvement of the current state-of-the-art
2) thoroughly investigate the literature and collect the information on potential biomarkers for the specified purpose
3) perform power calculations (based on realistic estimates of the expected performance) and establish a study plan, ideally discuss the approach with regulators, payers, and patient organizations to ease subsequent implementation
4) perform the study (which in our opinion may well be based on bio-banked samples) and, if everything goes well, demonstrate a significant improvement in diagnosis, prognosis, etc., as a result of applying the biomarker(s)
5) test and ideally demonstrate the benefit of implementing the biomarker in an appropriate intervention trial.

Such approaches, however, are quite time-consuming and expensive. As such, to implement such a strategy, the active support from funders and at least indirect support from publishers are required.

Additional discovery studies only seem justified if the studies published to date do not in fact allow addressing the specific need, for example, the biomarkers already described are of insufficient quality, clearly not fit for the purpose, etc. A discovery study that does not take into account all the available information (step 2) or for which no evident need (step 1) can be presented should be avoided.

However, as long as the competing solution, the underpowered and preliminary discovery study, is at least indirectly favored, it will be almost impossible for scientists to implement a truly useful approach in proteomic biomarker research.

5.2. Toward Drug Development

Current methods applied for the identification of potential drug targets underestimate the impact of the molecular complexity of disease, resulting in unexpected side effects and resistance to the intervention. Disease heterogeneity and highly complex molecular mechanisms underlying disease onset and progression are stumbling blocks in the selection of optimal drug targets. Proteomics has emerged as a promising tool to select ideal drug targets and bring personalized medicine closer to reality. The following considerations may facilitate drug discovery though the improvements in the identification of novel therapeutic targets:

1) extensive information on disease-associated molecular changes can be obtained through the application of protein-centered analysis of clinical specimens, supported by publicly available transcriptomics data and curated literature-mined data, as high phenotypic and molecular diversity of disease impede drug development and effective therapy
2) novel drug targets can be identified through linking the molecular changes to the disease aetiology via advanced bioinformatics tools, as part of a systems biology approach. This enables revealing affected molecular pathways and identifying molecules that cause the disease (being best suited targets)
3) selecting an appropriate model system, displaying molecular homology to the human disease will allow improved assessment of the impact of new drug targets in appropriate disease models (in vitro and in vivo).

It is foreseeable that such focus toward identification of valid and molecularly driven drug targets through the knowledge gained on disease-associated mechanisms (as indicated in the examples above), followed by validation in appropriate model systems will result in the identification of novel drug targets that have the potential to improve on the outcome of patients, and further support introducing personalized medicine.

6. Conclusion

When placing implementation as the main objective, the performance of the clinical proteomics field to date cannot be considered a full success. While the reasons for this moderate success and the obstacles presented in this manuscript are likely not complete, they certainly represent major issues. A thorough discussion with the aim to define the major problems and present possible solutions seems to be urgently required, otherwise the field of clinical proteomics may lose all credibility, due to not delivering. Considering the plethora of potential biomarkers reported so far, the technological advancements in the field, and the wide access to well-established biobanks, especially when aiming at the implementation, the focus should be now placed on performing the well-designed and well-powered validation studies. We are aware that our opinion expressed here may be biased and may not be shared by all readers, but we sincerely hope that this manuscript initiates a lively discussion on ways forward in implementing clinical proteomics in patient management.

Acknowledgements

M.F. and A.L. contributed equally to this work. The research presented in this manuscript was supported in part by the FP7 programs: "Clinical and System-Omics for the Identification of the Molecular Determinants of Established Chronic Kidney Disease" (iMODE-CKD, PEOPLE-ITN-GA-2013-608332), "Molecular Medicine" and "Systems Biology to

Identify Molecular Targets for Vascular Disease Treatment" (SysVasc, HEALTH-2013 603288); and by the H2020 programs: "Molecular Profiling of Bladder Cancer to Support Personalized Medicine" (BioMedBC, H2020-MSCA-IF-2016-752755) and "Multi-Omics Molecular Treatment Targets for Prostate Cancer" (PCaProTreat, H2020-MSCA-IF-2017- 800048); and the CliniMARK COST action (CA16113). The authors are grateful to Profs. Antonia Vlahou, Joost Schanstra, Christian Delles, Jan Staessen, and Theo Luider for critically evaluating the manuscript.

Conflict of Interest

H.M. is the cofounder and co-owner of Mosaiques Diagnostics. M.F. and A.L. are employed by Mosaiques Diagnostics.

Keywords

biomarkers, drug targets, mass spectrometry, personalized medicine, proteomics

Received: March 9, 2018
Revised: May 10, 2018
Published online: July 8, 2018

[1] W. Kolch, H. Mischak, A. R. Pitt, *Clin. Sci. (Lond)* **2005**, *108*, 369.
[2] H. Mischak, *Nephrol. Dial. Transplant.* **2015**, *30*, 532.
[3] H. Mischak, G. Allmaier, R. Apweiler, T. Attwood, M. Baumann, A. Benigni, S. E. Bennett, R. Bischoff, E. Bongcam-Rudloff, G. Capasso, J. J. Coon, P. D'Haese, A. F. Dominiczak, M. Dakna, H. Dihazi, J. H. Ehrich, P. Fernandez-Llama, D. Fliser, J. Frokiaer, J. Garin, M. Girolami, W. S. Hancock, M. Haubitz, D. Hochstrasser, R. R. Holman, J. P. Ioannidis, J. Jankowski, B. A. Julian, J. B. Klein, W. Kolch, T. Luider, Z. Massy, W. B. Mattes, F. Molina, B. Monsarrat, J. Novak, K. Peter, P. Rossing, M. Sanchez-Carbayo, J. P. Schanstra, O. J. Semmes, G. Spasovski, D. Theodorescu, V. Thongboonkerd, R. Vanholder, T. D. Veenstra, E. Weissinger, T. Yamamoto, A. Vlahou, *Sci. Transl. Med.* **2010**, *2*, 46ps42.
[4] J. M. Lee, J. J. Han, G. Altwerger, E. C. Kohn, *J. Proteom.* **2011**, *74*, 2632.
[5] F. Molina, M. Dehmer, P. Perco, A. Graber, M. Girolami, G. Spasovski, J. P. Schanstra, A. Vlahou, *Nephrol. Dial. Transplant* **2010**, *25*, 1015.
[6] K. Cisek, M. Krochmal, J. Klein, H. Mischak, *Nephrol. Dial. Transplant* **2015**, *31*, 2003.
[7] P. Lescuyer, D. Hochstrasser, T. Rabilloud, *J. Proteome Res.* **2007**, *6*, 3371.
[8] M. Krochmal, G. Kontostathi, P. Magalhaes, M. Makridakis, J. Klein, H. Husi, J. Leierer, G. Mayer, J. L. Bascands, C. Denis, J. Zoidakis, P. Zuerbig, C. Delles, J. P. Schanstra, H. Mischak, A. Vlahou, *Sci. Rep.* **2017**, *7*, 15160.
[9] F. L. Ren, H. Wu, Y. L. Lei, H. Y. Zhang, R. Liu, Y. Zhao, X. C. Chen, D. Q. Zeng, A. P. Tong, L. J. Chen, Y. Q. Wei, C. H. Huang, *Mol. Cancer* **2010**, *9*, 81.
[10] T. Yokoyama, T. Enomoto, S. Serada, A. Morimoto, S. Matsuzaki, Y. Ueda, K. Yoshino, M. Fujita, S. Kyo, K. Iwahori, M. Fujimoto, T. Kimura, T. Naka, *Int. J. Cancer* **2013**, *132*, 472.
[11] T. Matsubara, A. Mita, K. Minami, T. Hosooka, S. Kitazawa, K. Takahashi, Y. Tamori, N. Yokoi, M. Watanabe, E. Matsuo, O. Nishimura, S. Seino, *Cell Metab.* **2012**, *15*, 38.
[12] A. Latosinska, M. Mokou, M. Makridakis, W. Mullen, J. Zoidakis, V. Lygirou, M. Frantzi, I. Katafigiotis, K. Stravodimos, M. C. Hupe, M. Dobrzynski, W. Kolch, A. S. Merseburger, H. Mischak, M. G. Roubelakis, A. Vlahou, *Oncotarget* **2017**, *8*, 69435.
[13] H. Mischak, C. Delles, A. Vlahou, R. Vanholder, *Nat. Rev. Nephrol.* **2015**, *11*, 221.
[14] C. A. Borrebaeck, *Nat. Rev. Cancer* **2017**, *17*, 199.
[15] M. Frantzi, A. Latosinska, L. Fluhe, M. C. Hupe, E. Critselis, M. W. Kramer, A. S. Merseburger, H. Mischak, A. Vlahou, *Nat. Rev. Urol* **2015**, *12*, 317.
[16] D. M. Good, P. Zürbig, A. Argiles, H. W. Bauer, G. Behrens, J. J. Coon, M. Dakna, S. Decramer, C. Delles, A. F. Dominiczak, J. H. Ehrich, F. Eitner, D. Fliser, M. Frommberger, A. Ganser, M. A. Girolami, I. Golovko, W. Gwinner, M. Haubitz, S. Herget-Rosenthal, J. Jankowski, H. Jahn, G. Jerums, B. A. Julian, M. Kellmann, V. Kliem, W. Kolch, A. S. Krolewski, M. Luppi, Z. Massy, M. Melter, C. Neususs, J. Novak, K. Peter, K. Rossing, H. Rupprecht, J. P. Schanstra, E. Schiffer, J. U. Stolzenburg, L. Tarnow, D. Theodorescu, V. Thongboonkerd, R. Vanholder, E. M. Weissinger, H. Mischak, P. Schmitt-Kopplin, *Mol. Cell. Proteomics* **2010**, *9*, 2424.
[17] S. S. Roscioni, Z. D. de, M. E. Hellemons, H. Mischak, P. Zurbig, S. J. Bakker, R. T. Gansevoort, H. Reinhard, F. Persson, M. Lajer, P. Rossing, H. J. Heerspink, *Diabetologia* **2012**, *56*, 259.
[18] J. Siwy, J. P. Schanstra, A. Argiles, S. J. L. Bakker, J. Beige, P. Boucek, K. Brand, C. Delles, F. Duranton, B. Fernandez-Fernandez, M. L. Jankowski, M. Al Khatib, T. Kunt, M. Lajer, R. Lichtinghagen, M. Lindhardt, D. M. Maahs, H. Mischak, W. Mullen, G. Navis, M. Noutsou, A. Ortiz, F. Persson, J. R. Petrie, J. M. Roob, P. Rossing, P. Ruggenenti, I. Rychlik, A. L. Serra, J. Snell-Bergeon, G. Spasovski, O. Stojceva-Taneva, M. Trillini, H. von der Leyen, B. M. Winklhofer-Roob, P. Zurbig, J. Jankowski, *Nephrol. Dial. Transplant.* **2014**, *29*, 1563.
[19] J. P. Schanstra, P. Zurbig, A. Alkhalaf, A. Argiles, S. J. Bakker, J. Beige, H. J. Bilo, C. Chatzikyrkou, M. Dakna, J. Dawson, C. Delles, H. Haller, M. Haubitz, H. Husi, J. Jankowski, G. Jerums, N. Kleefstra, T. Kuznetsova, D. M. Maahs, J. Menne, W. Mullen, A. Ortiz, F. Persson, P. Rossing, P. Ruggenenti, I. Rychlik, A. L. Serra, J. Siwy, J. Snell-Bergeon, G. Spasovski, J. A. Staessen, A. Vlahou, H. Mischak, R. Vanholder, *J. Am. Soc. Nephrol.* **2015**, *26*, 1999.
[20] M. Lindhardt, F. Persson, G. Currie, C. Pontillo, J. Beige, C. Delles, H. von der Leyen, H. Mischak, G. Navis, M. Noutsou, A. Ortiz, P. L. Ruggenenti, I. Rychlik, G. Spasovski, P. Rossing, *BMJ Open* **2016**, *6*, e010310.
[21] J. Klein, C. Lacroix, C. Caubet, J. Siwy, P. Zurbig, M. Dakna, F. Muller, B. Breuil, A. Stalmach, W. Mullen, H. Mischak, F. Bandin, B. Monsarrat, J. L. Bascands, S. Decramer, J. P. Schanstra, *Sci. Transl. Med.* **2013**, *5*, 198ra106.
[22] E. M. Weissinger, J. Metzger, C. Dobbelstein, D. Wolff, M. Schleuning, Z. Kuzmina, H. Greinix, A. M. Dickinson, W. Mullen, H. Kreipe, I. Hamwi, M. Morgan, A. Krons, I. Tchebotarenko, D. Ihlenburg-Schwarz, E. Dammann, M. Collin, S. Ehrlich, H. Diedrich, M. Stadler, M. Eder, E. Holler, H. Mischak, J. Krauter, A. Ganser, *Leukemia* **2013**, *28*, 842.
[23] J. R. Yates, C. I. Ruse, A. Nakorchevsky, *Annu. Rev. Biomed. Eng.* **2009**, *11*, 49.
[24] S. Strohkamp, T. Gemoll, J. K. Habermann, *Proteomics* **2016**, *16*, 2519.
[25] D. Domanski, A. J. Percy, J. C. Yang, A. G. Chambers, J. S. Hill, G. V. C. Freue, C. H. Borchers, *Proteomics* **2012**, *12*, 1222.
[26] C. Pontillo, S. Filip, D. M. Borras, W. Mullen, A. Vlahou, H. Mischak, *Proteomics Clin. Appl.* **2015**, *9*, 322.
[27] M. Pejchinovski, D. Hrnjez, A. Ramirez-Torres, V. Bitsika, G. Mermelekas, A. Vlahou, P. Zurbig, H. Mischak, J. Metzger, T. Koeck, *Proteomics Clin. Appl.* **2015**, *9*, 453.
[28] H. Mischak, A. Vlahou, J. P. Ioannidis, *Clin. Biochem.* **2013**, *46*, 432.
[29] S. A. Carr, S. E. Abbatiello, B. L. Ackermann, C. Borchers, B. Domon, E. W. Deutsch, R. P. Grant, A. N. Hoofnagle, R. Huttenhain, J. M. Koomen, D. C. Liebler, T. Liu, B. MacLean, D. Mani, E. Mansfield,

H. Neubert, A. G. Paulovich, L. Reiter, O. Vitek, R. Aebersold, L. Anderson, R. Bethem, J. Blonder, E. Boja, J. Botelho, M. Boyne, R. A. Bradshaw, A. L. Burlingame, D. Chan, H. Keshishian, E. Kuhn, C. Kinsinger, J. S. H. Lee, S. W. Lee, R. Moritz, J. Oses-Prieto, N. Rifai, J. Ritchie, H. Rodriguez, P. R. Srinivas, R. R. Townsend, J. Van Eyk, G. Whiteley, A. Wiita, S. Weintraub, *Mol. Cell. Proteomics* **2014**, *13*, 907.

[30] G. Mermelekas, A. Vlahou, J. Zoidakis, *Expert. Rev. Mol. Diagn*. **2015**, *15*, 1441.

[31] M. Dakna, K. Harris, A. Kalousis, S. Carpentier, W. Kolch, J. P. Schanstra, M. Haubitz, A. Vlahou, H. Mischak, M. Girolami, *BMC Bioinformatics* **2010**, *11*, 594.

[32] H. Mischak, R. Apweiler, R. E. Banks, M. Conaway, J. J. Coon, A. Dominizak, J. H. Ehrich, D. Fliser, M. Girolami, H. Hermjakob, D. F. Hochstrasser, V. Jankowski, B. A. Julian, W. Kolch, Z. Massy, C. Neususs, J. Novak, K. Peter, K. Rossing, J. P. Schanstra, O. J. Semmes, D. Theodorescu, V. Thongboonkerd, E. M. Weissinger, J. E. Van Eyk, T. Yamamoto, *Proteomics Clin. Appl*. **2007**, *1*, 148.

[33] H. Mischak, J. P. Ioannidis, A. Argiles, T. K. Attwood, E. Bongcam-Rudloff, M. Broenstrup, A. Charonis, G. P. Chrousos, C. Delles, A. Dominiczak, T. Dylag, J. Ehrich, J. Egido, P. Findeisen, J. Jankowski, R. W. Johnson, B. A. Julien, T. Lankisch, H. Y. Leung, D. Maahs, F. Magni, M. P. Manns, E. Manolis, G. Mayer, G. Navis, J. Novak, A. Ortiz, F. Persson, K. Peter, H. H. Riese, P. Rossing, N. Sattar, G. Spasovski, V. Thongboonkerd, R. Vanholder, J. P. Schanstra, A. Vlahou, *Eur. J. Clin. Invest.* **2012**, *42*, 1027.

[34] H. Mischak, E. Critselis, S. Hanash, W. M. Gallagher, A. Vlahou, J. P. Ioannidis, *Am. J. Epidemiol*. **2015**, *181*, 635.

[35] E. Nkuipou-Kenfack, P. Zurbig, H. Mischak, *Proteomics. Clin. Appl*. **2017**, *11*, 1600104.

[36] FDA issues letter of support for urinary peptide-based biomarker to detect early stage of CKD. European Nephrology Portal **2016**, https://www.enp-era-edta.org/article/20.

[37] C. Pontillo, Zhang Z, J. Schanstra, L. Jacobs, P. Zuerbig, L. Thijs, A. Ramirez-Torres, H. J. L. Heerspink, R. Klein, T. Orchard, M. Porta, R. W. Bilous, N. Chaturvedi, P. Rossing, A. Vlahou, E. Schepers, G. Glorieux, W. Mullen, C. Delles, P. Verhamme, R. Vanholder, J. Staessen, H. Mischak, J. Jankowski, *Kidney Int. Rep*. **2017**, *2*, 1066 in press.

[38] C. Pontillo, L. Jacobs, J. A. Staessen, J. P. Schanstra, P. Rossing, H. J. L. Heerspink, J. Siwy, W. Mullen, A. Vlahou, H. Mischak, R. Vanholder, P. Zurbig, J. Jankowski, *Nephrol. Dial. Transplant.* **2017**, *32*, 1510.

[39] T. Kuznetsova, H. Mischak, W. Mullen, J. A. Staessen, *Eur. Heart J*. **2012**, *33*, 2342.

[40] Z. Zhang, J. A. Staessen, L. Thijs, Y. Gu, Y. Liu, L. Jacobs, T. Koeck, P. Zurbig, H. Mischak, T. Kuznetsova, *Int. J. Cardiol*. **2014**, *176*, 158.

[41] Z. Y. Zhang, L. Thijs, T. Petit, Y. M. Gu, L. Jacobs, W. Y. Yang, Y. P. Liu, T. Koeck, P. Zurbig, Y. Jin, P. Verhamme, J. U. Voigt, T. Kuznetsova, H. Mischak, J. A. Staessen, *Hypertension* **2015**, *66*, 52.