Quo vadis, biomarkers?

The unprecedented progress of medical therapy over the last few decades is associated with the advancement of understanding of molecular mechanisms of human diseases. Laboratory medicine is an integral part of this effort as it is not only instrumental in studies that are defining molecular bases of different disorders, but also provides biomarkers that aid the selection of treatment for individual patients [1]. In fact, the entire concept of patient-tailored therapy would not be possible without simultaneous advances of research on biomarkers. Yet both the development of new therapies and new biomarkers is still lagging behind the demands of a society plagued by an epidemic of chronic incurable disorders [2].

In the last decade, the research into biomarkers has been compared to the quest for the Holy Grail as it has not yet delivered on its promise. The at first hypothetical usefulness of biomarkers for screening and prediction of risk of disease, diagnosis, staging, prediction or monitoring of treatment response, and for monitoring compliance has since been widely and increasingly accepted. Biomarkers have long been hailed as the key to better patient care and lower medical costs, as well as catalysts for personalized medicine [3]. Despite growing interest and investments, the number of biomarkers receiving United States Food and Drug Administration (FDA) clearance has, however, declined substantially over the past 10 years to less than one protein biomarker per year [4]. In contrast to the huge amount of literature comprising more than 150,000 papers documenting thousands of putative biomarkers, less than 100 biomarkers have been validated for clinical practice being portrayed as a “drop in the ocean” [5]. It has been highlighted that the journey of a new biomarker from the bench to the bedside is hence long and challenging, and every step in this pipeline must be meticulously planned and properly executed [6]. In particular, a review of the literature on translational research in oncology has revealed that most of the 939 publications on prognostic factors for patients with breast cancer that have appeared over a 20-year period were based on research assays with poor evidence of robustness or analytical validity [7]. These facts led journal editors to ask for more robustness of the analytical techniques used for quantification of novel, putative biomarkers [8], since problems such as data manipulation, poor experimental design, reviewer’s bias and over-interpretation of results are reported with increasing frequency [9].

Most biomarkers are proteins and the vast majority of methods used for biomarker determination are based on antibodies and related immunoassays. Recently, Rifai and colleagues have emphasized that caution should be adopted when using commercially available immunoassays to avoid the risk of wasting money and time “if the assay used in the validation study is of poor quality and does not measure the stated analyte with the expected sensitivity and specificity” [10]. In this issue of the journal, Prassas and Diamandis add fuel to the fire by alerting translational researchers on the pitfalls and poor quality of many commercially available ELISA kits and inviting all stakeholders to take into serious consideration the need for the creation of independent bodies for standardized antibody validation. In fact, the authors stress the point that many companies obtain their own reagents (antigens as well as antibodies) from external suppliers “without a rigorous validation”. In addition, and we fully agree with the authors, it is extremely difficult to discern the roles of the different parties involved (suppliers, manufacturers or distributors) [11]. Prassas and Diamandis document the consequences of using poorly validated reagents inviting translational researchers to change the lack of concern and false belief that this represents a trivial issue. The first “take-home” message, therefore, is that the evaluation and validation of analytical methods is still an essential step in the pipeline of biomarker development [12]. However, other fundamental issues should be taken into consideration.

It has been already emphasized that a major impediment to the progress in the hunt for biomarkers is the lack of standardization of how specimens are collected, handled and stored thus calling for the adoption of harmonized guidelines [13]. In addition, the recent shift to testing for multiple biomarkers (multiplex profiling), particularly when obtained with high-throughput technologies, such as for proteomics and gene profiling, makes increasingly important the use of sound statistical/bioinformatics methods, as well as the need of validation trials with unbiased study designs [14].
Table 1 summarizes current evidence on the obstacles in translating biomarker research that should be found both in pre-, intra- and post-analytical steps.

Currently, metastatic cancer still represents in most cases an incurable disease. Therefore, medical oncology has been in the forefront of the research on new agents and also new biomarkers as there is an unmet medical need for new comprehensive treatment approaches in the treatment of cancer [15]. The development of new targeted agents is critically dependent on the identification of predictive biomarkers that select patients for the particular therapy. It is now evident that neoplastic diseases like breast cancer that have been hitherto considered as a single entity in fact represent a spectrum of many different malignant disorders, with important repercussions for clinical practice. For example, treatments targeting human epidermal growth factor receptor-2 (HER-2), e.g., trastuzumab, that have changed the natural history and transformed the management of HER-2-positive breast cancer are active only in the particular subset of patients with tumors which overexpress HER-2 representing just about 15% of all breast cancer patients [16]. Without the identification of a predictive biomarker the activity of anti-HER-2 therapy would be diluted by the lack of efficacy of this approach in the majority of patients with tumors not overexpressing HER-2, and depending on the design these agents might have been considered ineffective in trials that would not incorporate a predictive biomarker. While in the case of trastuzumab or other anti-HER-2 agents the development of this treatment approach was intimately linked to predictive biomarkers, for other targeted agents this path has not been so straightforward. We may consider the example of monoclonal antibodies targeting another member of HER family of receptors, epidermal growth factor receptor (EGFR; HER-1). In contrast to HER-2 and trastuzumab, it was evident already in early clinical trials that the activity of anti-EGFR antibody, cetuximab, is not linked to the expression of EGFR on tumor cells [17]. For several years, the only reliable predictive biomarker of cetuximab activity was the manifestation of skin toxicity [18] before the evidence emerged that this therapy is active only in patients with tumors that do not harbor mutations of the RAS gene. The realization that the activity of some agents that have subsequently transformed management of patients with cancer like trastuzumab or anti-HER-2 antibodies cetuximab or panitumumab might have been missed if the setting of the trials was different is behind the current initiative of the National Cancer Institute of the United States to create a registry of exceptional responders to novel anticancer agents. However, listing of responses represents just the first step in this effort. Agents that are active only in a small proportion of individuals are unlikely to be used in clinical practice if there is no way to identify patients that are likely to respond to a given treatment. The crucial task of finding these biomarkers is the realm of laboratory medicine. In other words, there will be no new drugs for these rare responders without the simultaneous advent of new biomarkers.

In this issue of the journal, Diamandis adds another piece in the puzzle of biomarker development and utilization [19], inviting us to avoid the risk to “Throw out the baby with the bath water”. The evidence that some
biomarkers presenting low clinical sensitivity, despite very high specificity may discriminate few patients from normal subjects and benign diseases leads Diamandis to make an intriguing proposal that resembles the program of the National Cancer Institute to catalog “exceptional responders” in cancer trials. The proposal is to catalog cancer biomarkers which can be used in “small groups of informative patients to identify optimal treatments, institute optimal monitoring or to assess the prognosis of such patients”. However, possibly the most interesting goal is to investigate if such tumor marker alterations are associated with specific molecular changes thus providing a mechanistic rationale for their elevation in biological samples. This, in turn, may highlight the need to look at the biomarker pipeline as a two-way road: not only from bench to bedside but also from bedside to bench involving multiple stakeholders, including laboratory professionals [20]. In fact, creating the registry of “rare” responders and “rare” biomarkers are complementary projects. The catalog of rare responders would be just a collection of interesting case reports without the identification of biomarkers that would help in understanding why that particular patient responded. Similarly, all the efforts aimed at finding rare biomarkers would remain a scholar exercise if these findings do not open the way for new therapies. Moreover, the detection of rare biomarkers should not be limited only to tumor diagnosis, but should also include biomarkers predicting or detecting rare toxicities. These “rare” biomarkers may also offer an invaluable insight into the pathogenesis of the disease or complications of therapy.

In conclusion, the comments by Prassas and Diamandis as well as the proposal by Diamandis highlight serious issues facing the development of new drugs and diagnostic approaches, in particular in cancer medicine. It has to be realized that laboratory medicine is currently a crucial partner of medical oncology in promoting patient-tailored therapeutic approaches, and that finding the appropriate predictive biomarker may be as important as discovering the new effective drug. From a historical perspective, similarly to the proposal to find rare responders to new agents that could potentially transform the practice of medical oncology, the efforts aimed at the identification of rare biomarkers could, sometime in the future, be viewed to represent an important milestone in the field of laboratory medicine.

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*Corresponding author: Prof. Mario Plebani, Department of Laboratory Medicine, University Hospital of Padua, Padua, Italy, Phone: +39 498212792, Fax: +39 49663240, E-mail: mario.plebani@unipd.it

Bohuslav Melichar: Department of Oncology, Palacký University Medical School and Teaching Hospital, University Hospital, Olomouc, Czech Republic