

# Cancer molecular pathobiology in the clinics: Concluding remarks

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This interesting issue in which the Institute of Oncology “Prof. Dr. Ion Chiricuta” of Cluj and the Cancer Institute “G. Paolo II” of Bari approach the problem of cancer biomarkers from different perspectives represents an attempt to provide a novel view of biological, clinical and methodological innovative aspects in this field. In fact, there is a critical need for reliable biomarkers in cancer in order to improve (i) early detection, (ii) accuracy of prognosis, (iii) patient stratification for treatments, and (iv) monitoring of tumor response and relapse. Although technological advances in the field of genomics, metabolomics, and proteomics have resulted in the identification of many biomarker candidates, only a few of them have been approved and display a value in clinical practice. This is particularly true for biomarkers suitable for early diagnosis of cancers, despite the considerable efforts devoted to this goal.

In the field of diagnosis, one important challenge is getting biomarkers specific enough to avoid false positive conclusions that lead to unnecessarily expensive (imaging) and invasive (biopsies) interventions. Although novel technologies have produced detailed information on the molecular aspects of cancer biology, for the most part, the specificity displayed by single or multiple biomarkers is insufficient to allow the assays to be used in large populations. Very sensitive techniques to address specific hallmarks of cancer and combined approaches (metabolomics and imaging, for instance) need to be improved in the future in order to succeed in this objective. Next-generation sequencing techniques and especially RNAseq provide data about cancer-specific splice variants that can be developed as

very useful biomarkers, either by detection of the variants as circulating DNA or by using antibodies when the splice variant results in novel epitopes in transmembrane or soluble proteins.

In terms of prognosis and prediction of response, the situation is more favorable because of access to tumor biopsies, which allows the evaluation of different biomarkers at the protein, RNA or DNA levels. A number of signatures are commercialized or under evaluation in large clinical trials and will help clinicians to make therapeutic decisions. In the case of gene profiling in breast cancer, patients who can benefit from adjuvant chemotherapy can be selected based on a high risk of local relapse/ metastasis or to exclude low-risk patients from unnecessary therapeutic intervention.

In the era of evidence-based medicine, there is an emergence of biomarkers predicting the response to targeted therapy. These biomarkers allow the selection of the patients who would or would not benefit from a given treatment. Determination of HER2 expression or KRAS mutations are mandatory for selecting patients to be treated with Trastuzumab or Cetuximab in breast or colorectal cancers, respectively. Looking at the expression of immunoregulatory molecules at the surface of tumor cells or immune infiltrates by IHC will be a requisite to treating patients with novel neutralizing immunomodulatory antibodies recognizing these proteins. Obviously, many pharmaceutical companies develop companion diagnostics together with new drugs and invest a great deal of money in identifying pharmacokinetic, toxicological and predictive biomarkers in both preclinical settings and during early and late clinical trials.

Although repeated biopsies are very useful to monitor biomarkers after tumor relapsing, the use of non-invasive methods might be preferable. These non-invasive techniques are ideal for detecting as early as possible the relapse of tumors or to evaluate the response to drugs (and modifying the treatment when disease is progressing).

Noninvasive approaches include: (i) the search and quantification of molecules (nucleic acids, proteins, sugars, lipids, and metabolites) in biofluids (serum, plasma, saliva, urine, fecal water) by many different techniques and (ii) the use of imaging to visualize the presence of biomarkers in tumor cells or its microenvironment.

A lot of attention has been paid in the last years to the nucleic acids in fluids as cancer biomarkers. Potential nucleic acids biomarkers include not only DNA, but also RNA: microRNAs (miRs) and more recently long non-coding RNAs (lncRNAs) protected from degradation by its packaging in microparticles. I will briefly discuss the potential of these biomarkers.

Cell-free circulating DNA in different fluids (plasma, serum or urine) is becoming one of the most powerful biomarkers for the prognosis and monitoring of many cancers. Circulating tumor DNA displays the mutations, gene fusions, structural rearrangements, and epigenetic alterations (methylation), as well as microsatellite instability and loss of heterozygosity of primary tumors. Different technologies have been developed to detect circulating tumor DNA harboring tumor-specific DNA aberrations. These techniques include the massive parallel sequencing of plasma DNA, beads, emulsion, amplification, and magnetics (BEAMing) assay, digital PCR, short oligonucleotide mass analysis, or bidirectional pyrophosphorolysis-activated polymerization (bi-PAP). Importantly, the sequencing of plasma DNA has been shown to accurately identify markers of resistance to targeted drugs. Although these techniques are used mainly for detecting recurrent genetic alterations (point mutations or translocations), wide access to massive sequencing to characterize the tumors of patients will conduct in the future to the use of personalized biomarkers.

Many studies have reported circulating cell-free miRs as promising biomarkers for many cancer types. Actually a series of "miR signatures" have been reported to predict the prognosis of some cancers and have shown to have good potential for cancer screen-

ing. Given that blood cells are a major source of circulating miRs, changes in the number of blood cells or hemolysis can critically affect their levels in patients. Therefore, the different signatures need to be carefully examined in this context in order to be validated in clinics.

lncRNAs are very attractive targets for cancer biomarkers because their number is rapidly expanding and because they show high tissue specificity when compared with protein-coding RNAs. Some lncRNAs are expressed differentially in human cancer, and this opens an avenue for the identification of new biomarkers, either by quantification or detection of mutated forms. Importantly, lncRNA PCA3 has been approved recently by the FDA to help determine the need for repeat prostate biopsies.

It is crucial to assess the clinical usefulness of nucleic acids as biomarkers in large prospective studies at different stages of the disease. The performances of these biomarkers need to be compared with the standards methods used to monitor prognosis, follow-up, response to therapy, and tumor relapse.

Cancer is a very complex disease. Not only does the biology of cancer cells change during the different steps of progression and subsequently the expression of biomarkers, but also we need to take into account the tumor heterogeneity and the crucial role of tumor microenvironment. With this complexity in mind, it seems very difficult to develop single cancer biomarkers. Over the next years, a personalization of cancer biomarkers will be needed.

In order to be implemented in clinics, a biomarker requires very high standards of efficiency and impact in patient care. These standards include sensitivity and specificity, reproducibility, safety, and cost efficiency. To reach these standards, it will be crucial over the next few years to implement multidisciplinary approaches linking academic and industrial teams to succeed in the validation, qualification and commercialization of biomarkers. This collaboration will provide access to high-quality retrospective cohorts of samples and the implementation of prospective cohorts appropriate to evaluate a potential biomarker.

With this in mind, we have to examine with an even greater interest the translational collaboration between cancer institutes. This is perhaps the only means to collect a large and high quality series of specimens required for such a complicated approach.