



Towards The Biomarker-Driven Development and Their Global Impact in the Evidence-Based Clinical Practice of Personalized and Precision Healthcare Services

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Drug development continues to move in the direction of the development of clinical practice as applicable to **Personalized and Precision Medicine (PPM)**, - where ideally the most effective therapy or treatment is determined by the genetic makeup of the patient. PPM, an upgraded healthcare approach that systematically uses patient and/or persons-at-risk data to inform individualized treatment decisions, has emerged as potentially transformative - offering the promise of superior treatment outcomes for most of the clients, including patients and/or persons-at-risk. PPM-related resources are supported by significant advances in **biomarker** testing and then in the validation to be used further in daily clinical practice (**Fig. 1**).

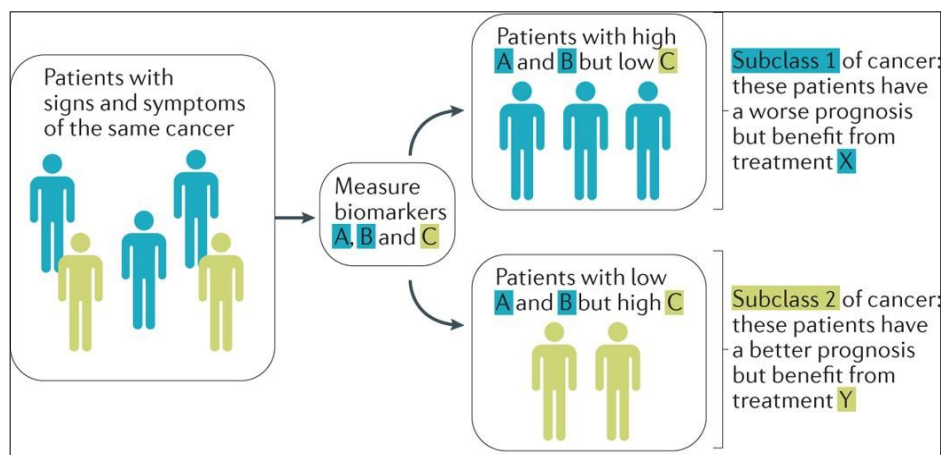


Fig 1: Biomarker development in the PPM-related era

PPM relies on validated biomarkers with which to better classify patients by their probable disease risk, prognosis and/or response to treatment. Although affordable 'OMICS'-based technology has enabled faster identification of putative biomarkers, the validation of biomarkers is still stymied by low statistical power and poor reproducibility of results PPM, personalized & precision medicine;

The source: Vargas, A., Harris, C. Biomarker development in the precision medicine era: lung cancer as a case study. *Nat Rev Cancer* 16, 525–537 (2016). <https://doi.org/10.1038/nrc.2016.56>

Yet, the promise of PPM cannot be realized if clients do not have access to the biomarker testing required to determine their eligibility for PPM-based treatments.

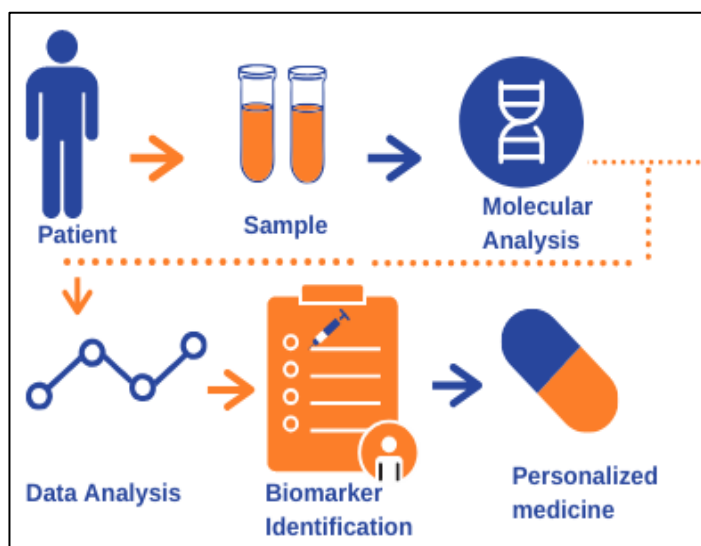


Fig 2: Role and impact of biomarkers in daily PPM-driven clinical practice

To develop PPM, the need to identify various disease stages of the patients is facilitated by biomarkers, which are mostly dictated by OMICS-based principles. In this context, precision diagnostics is a collection of techniques to analyze the biomarkers in the genes and proteins of biological entities. Those techniques are used to diagnose and monitor disease, identify risk, suggest which therapies are most suitable for individual patients. To achieve PPM for each patient or person-at-risk, the potential biomarkers need to be identified, verified, evaluated and validated.

PPM, personalized & precision medicine; OMICS, any of several areas of biological study defined by the investigation of the entire complement of a specific type of biomolecule or the totality of a molecular process within an organism. OMICS ends with -omics, such as genomics, proteomics, or metabolomics. Overall, the objective of OMICS is to identify, characterize, and quantify all biomolecules that are involved in the structure, function, and dynamics of a cell, tissue, or organism.

The source: Varalaxmi Reddy. Using AI To Identify Biomarkers That Facilitate Personalized Medicine. November 25, 2019 <https://www.proxzar.ai/blog/using-ai-to-identify-biomarkers-that-facilitate-personalized-medicine>

The area of **PPM** as an upgraded model of the healthcare services and thus an area of daily clinical practice, that involves the use of measuring **biomarkers** in clinical samples, is an area of high clinical interest (**Fig. 2**).

Tremendous efforts have been made to date to discover biomarkers of the next step generation for use in clinical practice, but, unfortunately, a rate of implementing of biomarkers into clinical practice is still rather low (1,2,6,14).

There are still many open questions in data-analytic research pertaining to biomarker development in the era of PPM, OMICS-technologies, Bioinformatics and IT-based resources, and Big Data. Among them is the question of what constitutes best practice for the extraction of prioritized lists of candidate biomarkers to be used in the right way in daily clinical practice and drug discovery.

Biomarkers - and the assays used to detect, measure, and characterize them - are the cornerstone of PPM, which aims to match the right patient with the right drug at the right dose and time to optimize treatment impact and patient outcome. So, biomarkers are considered to be essential and crucial for the development of PPM and PPM-driven technologies, and can thus be used in the immediate clinical practice (such as determining what devices or drugs are the best fit for patients, depending on the presence or absence of certain biomarkers) as a generation of the ready-to-be-used monitoring tools (endowing with diagnostic, predictive and prognostic resources) (**Fig.3 A, B**), in a broad scope of clinical settings to facilitate medical product development and inform patient care decisions, as well as for drug development (for example, patient selection) in the drug design-inspired biotech-driven translational research and applications (3,6,10-14). The latter is much illustrated by explosive bursts at the global worldwide markets (**Fig. 4**).

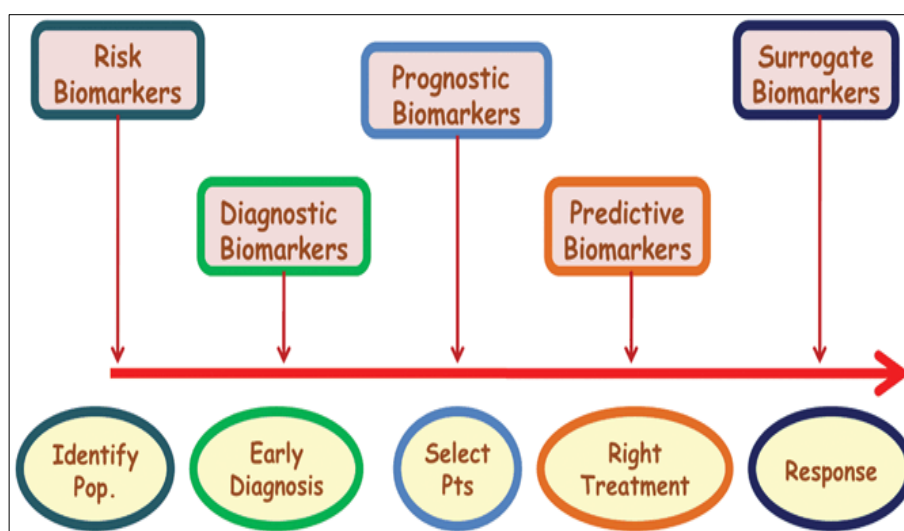


Fig 3A: Biomarkers as Diagnostic, Risk, Prognostic, Predictive, and Surrogate Endpoints

Biomarkers have several clinical applications in practice, including risk assessment for disease recurrence or early diagnosis in healthy population. After the advent of targeted therapies, a growing interest has been focused on their potential role as prognostic, predictive, and surrogate endpoints, in order to promote PPM-driven strategies. The introduction of molecular biomarkers in clinical practice has radically changed the natural history of some tumors and autoimmune disorders, allowing many patients and persons-at-risk to receive an individualized treatment.

PPM, personalized & precision medicine

The source: Francesco Passiglia, Giuseppe Cicero, Marta Castiglia. Chapter - Biomarkers as Prognostic, Predictive, and Surrogate Endpoints In: Targeted Therapies for Solid Tumors, Springer Nature, 2015 ISBN: 978-1-4939-2046-4

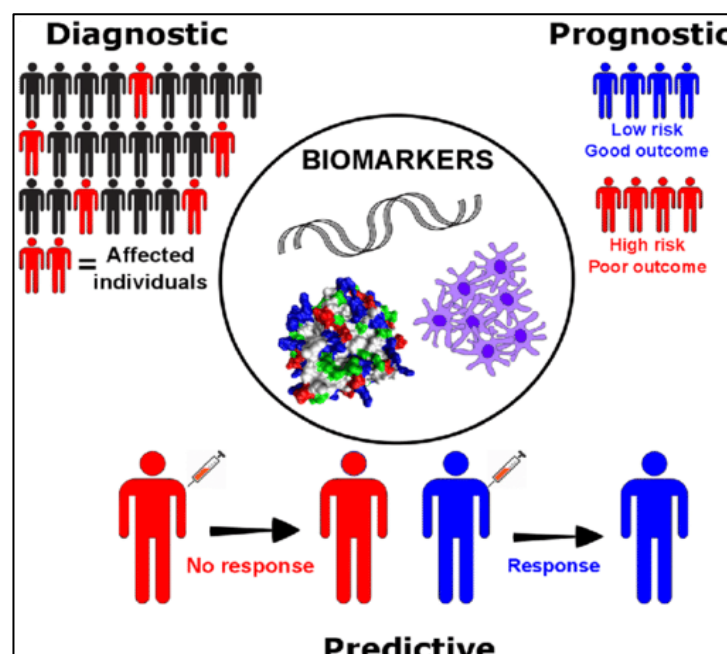


Fig 3B: Clinical applications of cancer biomarkers

Genetic, protein and cellular components can serve as diagnostic, prognostic and/or predictive biomarkers of cancer. Those molecules including circulating tumor DNA and RNA, proteins, tumor and immune cells are emerging as convenient and accurate liquid biomarkers of cancer. Diagnostic biomarkers are used to identify and detect presence of cancer in individuals, prognostic biomarkers provide information on disease progression and expected outcomes, and predictive biomarkers forecast the likely benefit of a specific treatment. Circulating cancer biomarkers provide invaluable information on cancer detection and diagnosis, prognosticate patient outcomes, and predict treatment response. In this era of effective molecular targeted treatments and immunotherapies, there is now an urgent need to implement use of these circulating biomarkers in the clinic to facilitate personalized therapy.

PPM, personalized & precision medicine

The source: Su Yin Lim et al. Liquid biomarkers in melanoma: detection and discovery. Molecular Cancer (2018) 17:8, PP 1-14

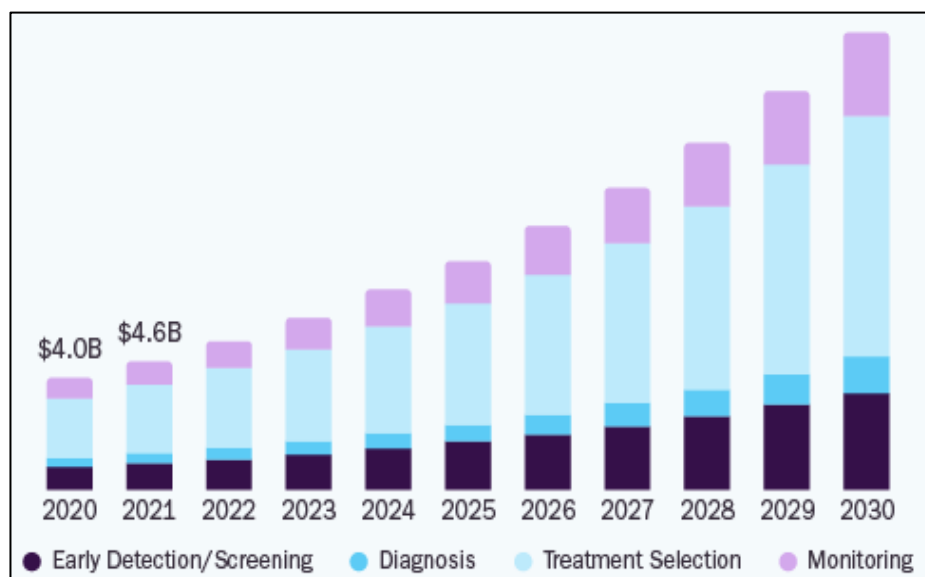


Fig. 4: U.S. Personalized & Precision Biomarkers Market size, by application, 2020-2030 (USD billion)

The global PPM biomarkers market size was estimated at USD 11.76 billion in 2021. It is expected to expand at a compound annual growth rate (CAGR) of 15.51% from 2022 to 2030. The growth can be attributed to the increasing demand for PPM, the increasing prevalence of chronic diseases, and technological advancements in the diagnosis and detection of chronic diseases. The growth is further propelled by lifesaving benefits offered by biomarkers, which include the ability to detect health conditions in their early stages, improve patient survival, and reduce treatment costs.

PPM, personalized & precision medicine; GAGR, compound annual growth rate

Modern diagnostic techniques are accelerating the shift toward PPM-related care for many ailments. With the aid of those techniques, physicians can identify and measure a variety of biomarkers to categorize patients and/or persons-at-risk into subgroups that differ in their tendency. This includes client's vulnerability to develop a disease or pre-illness, their personalized mutation to develop a disease even after successful treatment, and their likelihood of responding to a particular treatment. And technological advancements in the development of biomarkers thus witnesses a rapid rise as the demand for PPM grows substantially. Thus, to ensure client (patient or persons-at-risk) access to the new technologies, a reimbursement framework needs to be put in place to reflect the changing healthcare landscape. This will potentially encourage manufacturers to invest in new products as reimbursement policies which have a direct impact on the development and growth of biomarker-driven families of diagnostic, predictive and prognostic tools.

The value of biomarkers - characteristics that are assessed as indicators of physiological and/or pathogenic biological processes, or responses to an intervention - is broadly appreciated, but

the number of qualified and validated biomarkers is small. In this sense, among the main challenges to implementation of PPM into routine medical practice is a knowledge gap of professionals about biomarkers and biomarkers-driven tools to be used by the practitioners in their daily work. Moreover, biomarkers are useful for enrichment in regular clinical trials and identifying the “right” patients to enroll in clinical trials, whilst acting their crucial role as key contributors to drug design, drug discovery and drug development success as a whole. Biomarkers are used in drug development to help define mechanisms of action, drug target selection, patient selection, efficacy assessment, molecular pathways leading to disease, etc (2). As the use of biomarkers continues to expand across the research and care continuum, therapeutic developers are tasked with integrating clinical design and execution with a clear biomarker-informed development plan (**Fig.5**).

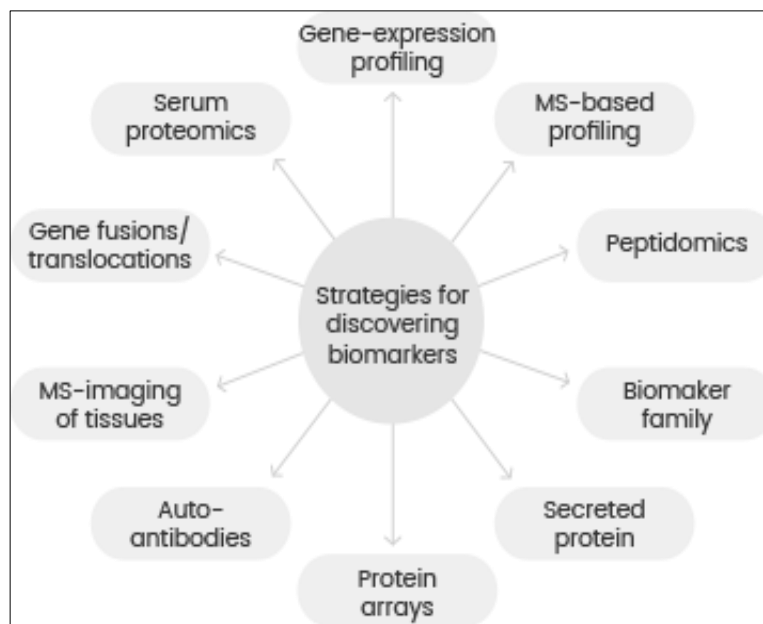


Fig. 5: Strategy for biomarker discovery

An ideal biomarker would satisfy the following properties: it should be either binary (i.e., present or absent) or quantifiable without subjective assessments; the result should be generated by an assay that is adaptable to routine clinical practice and has a timely turnaround (i.e., in a matter of days rather than weeks); the biomarker assay should be sensitive and specific; and most importantly, the biomarker should be detectable using easily accessible specimens. Meanwhile, the introduction of technologies such as mass spectrometry, peptidomics, secreted protein and biomarker-family approach, and protein and DNA/RNA arrays, combined with our understanding of the human genome, has led to renewed interest in the discovery of novel biomarkers. Despite the fact that new technologies and strategies often fail to identify well-established biomarkers, those technological advances have the capacity to revolutionize biomarker discovery.

The source: <https://www.sinobiological.com/resource/cancer-biomarker/discovery>

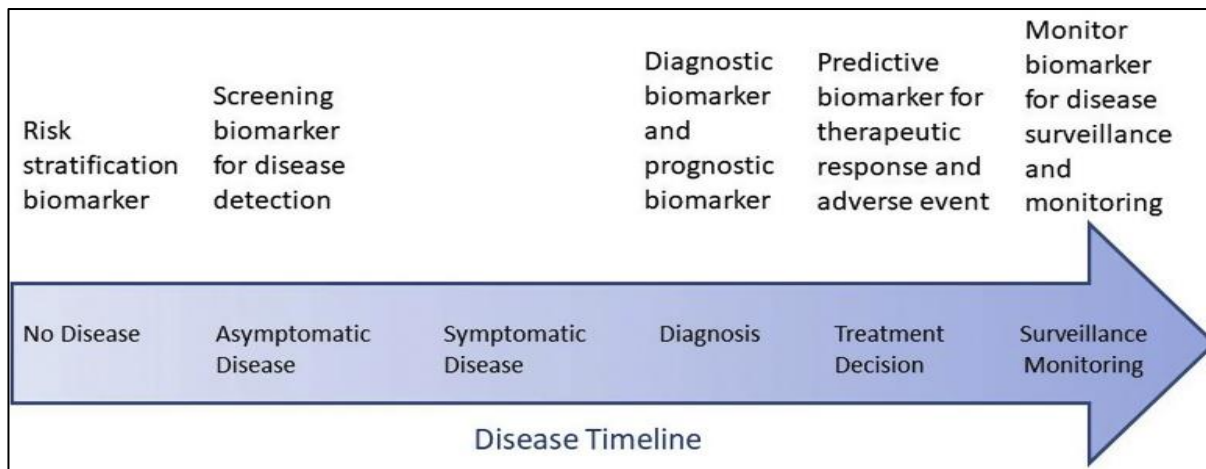


Fig. 6A: Application of exploratory and use of biomarkers in relation to the course of disease

Biomarkers have various applications, such as risk estimation, disease screening and detection, diagnosis, estimation of prognosis, prediction of benefit from therapy, and disease monitoring. The most common biomarkers are cancer-associated proteins, gene mutations, deletions, rearrangements, and extra copy numbers of genes. These molecules are sometimes secreted into the circulation and so may be detected by blood-based assay, whereas others are present in cancer cells and so require a biopsy to obtain tissue for testing

The source: Fang-Shu Ou et al. Biomarker Discovery and Validation: Statistical Considerations. Journal of Thoracic Oncology, Volume 16, Issue 4, 2021, Pages 537-545, ISSN 1556-0864, <https://doi.org/10.1016/j.jtho.2021.01.1616>.

A biomarker might be either a molecule secreted by a cell or a tumor, or it can be a specific response of the body to the presence of cancer. For instance, panels of genetic, epigenetic, proteomic, glycomic, and imaging biomarkers can be used for cancer diagnosis, prognosis and epidemiology. Consequently, biomarkers are not yet ready for routine use due to challenges in their clinical validation for pre-early (subclinical or pre-illness) disease detection, diagnosis and monitoring to improve long-term survival of patients. Molecular biomarkers are used together with clinical information to achieve PPM to customize prevention, screening, and treatment strategies to a group of patients with similar characteristics. As such, it is never too early to identify a defined pathway for taking an exploratory biomarker all the way through to diagnostic approval if data supports its use (**Fig. 6A, B**).

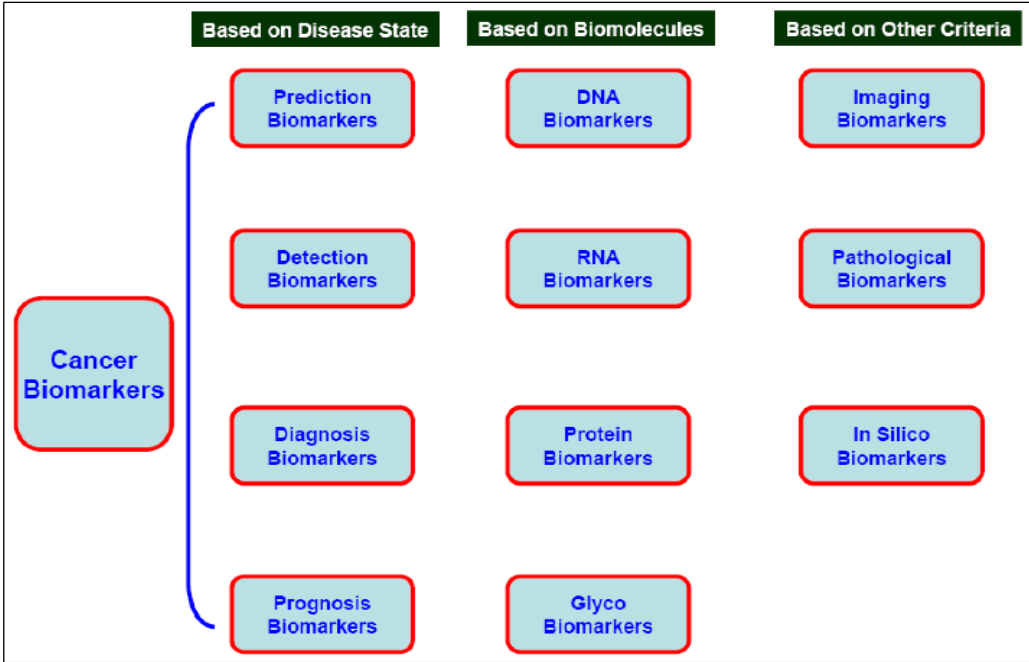


Fig. 6B: Criteria-based classification of biomarkers

Biomarkers offer great potential for improving management of chronic conditions, hereditary disorders and cancer at every point from screening and detection, diagnosis, staging, prognosis and monitoring, and assessment of treatment response. Clarification of the definitions of different biomarkers and a better understanding of their appropriate application could result in substantial benefits. For clinical implications, a biomarker needs to be validated in different institutes and in a large number of samples followed by approval from the FDA.

The FDA/NIH Leadership Council identified harmonization of biomarker-related terms as a priority to facilitate improved communication and shared understanding of terms related to biomarker development and utility among stakeholder groups.

The source: Mishra, A.; Verma, M. Cancer Biomarkers: Are We Ready for the Prime Time? *Cancers* 2010, 2, 190-208. <https://doi.org/10.3390/cancers2010190>

In general, biomarkers can indicate a variety of health or disease characteristics, including the level or type of exposure to an environmental factor, genetic susceptibility, genetic responses to exposures, markers of subclinical or clinical disease, or indicators of response to therapy. Thus, a simplistic way to think of biomarkers is as indicators of disease trait (risk factor or risk marker), disease state (subclinical or clinical), or disease rate (progression) (4,14).

Conceivably relevant biomarkers can be used to define subgroups of patients, and a patient's subgroup affiliation can be incorporated into evidence-based medical decisions. The most common biomarkers are cancer-associated proteins, gene mutations, and extra copy numbers of genes. These molecules are sometimes secreted into the circulation and so may be detected by blood-based assay, whereas others are present in cancer cells and so require a biopsy to obtain tissue for testing.

The contribution of OMICS technologies to PPM is via the identification of relevant biomarkers. In a first step, OMICS technologies allow generating vast amounts of data on particular molecules in individuals with a specific condition. Data are then analyzed to determine whether particular biomarkers are associated with the occurrence of the disease or, perhaps, with a given prognosis, or even with a certain response to a defined therapeutic intervention. The human biomarker database can be accessed online and the inter-disease relationships may be helpful in understanding the molecular mechanisms of diseases. To our knowledge, this is one of the first approaches to classify diseases based on biomarkers. And at the end of several rounds of analytical and clinical validation, biomarkers may be approved to be used in the clinic. Biomarkers are extremely important in cancer research and **personalized and precision oncology (PPO)**; they are crucial for risk assessment, screening, differential diagnosis, prognosis determination, prediction of disease recurrence and response to therapy, and progression monitoring [6,17,18]. With cutting-edge OMICS-based assays, as well as improved bioinformatics tools, the evolution of biomarkers to reliably assess the results of cancer mitigation and therapy is now possible. Looking forward, a urine or a serum test for each stage of cancer may possibly drive clinical decision making, complementing, or even replacing presently available invasive methods [6,18].

Due to the individualization of cancer therapy, the identification of cancer- and oncology-specific biomarkers has become a foremost goal for cancer researchers (6,18). A wide array of potential cancer biomarkers is used to track disease development and progression or response to therapy (**Fig. 7**).

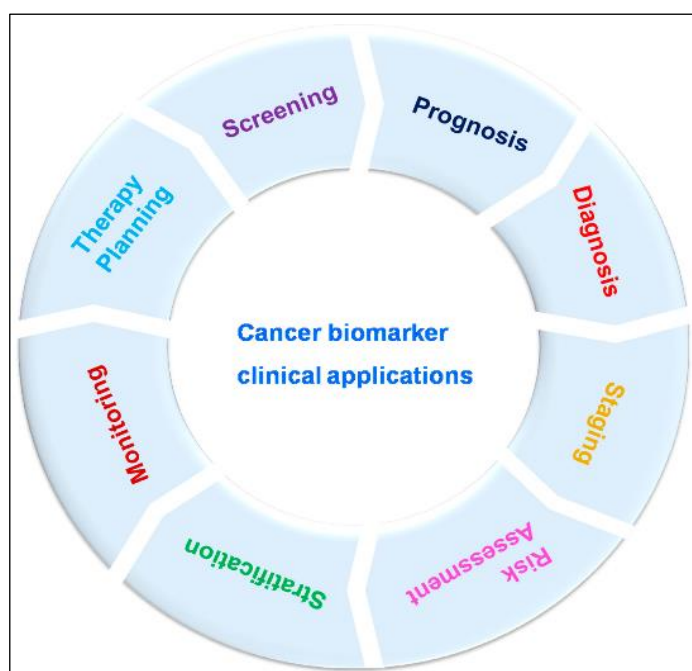


Fig. 7: Cancer biomarker clinical applications

Cancer markers are molecules of biologic emergence found in blood, tissues, various body fluids such as urine, cerebrospinal fluid, or different body tissues that are elevated is the indicative of an abnormal disease or condition with cancer. Human body responsiveness to any therapy can be observed and regulated through biomarkers. Cancer biomarkers can be utilized for cancer

patient evaluation in different clinical levels, as well as disease screening, prognosis, diagnosis, staging, risk assessment, stratification, therapy planning and monitoring. Biomarkers also play potential role in differentiating benign and malignant tumors and one type of malignancy from another type; specific biomarkers are helpful in unique settings; other biomarkers can be involved in multiple settings. Biomarkers could be utilized as stimulus changers or prognostic factors for unique type of therapy, as well as to select the effective type of treatment.

The source: Rao Bommi, J.; Kummari, S.; Lakavath, K.; Sukumaran, R.A.; Panicker, L.R.; Marty, J.L.; Yugender Goud, K. Recent Trends in Biosensing and Diagnostic Methods for Novel Cancer Biomarkers. *Biosensors* 2023, 13, 398. <https://doi.org/10.3390/bios13030398>

Predictive biomarkers for instance, cancer biomarkers such as **predictive** one, indicate the likely effect of a specific targeted therapy on the patient and can guide treatment decisions. Among the latter: (i) HER2 positivity/activation which predicts response to trastuzumab in breast cancer; (ii) KRAS-activating mutations which are associated with resistance to EGFR inhibitors (e.g., cetuximab) in colorectal cancer (CRC); (iii) BCR-ABL1 chromosomal alteration, which can predict positive response to treatment with tyrosine kinase inhibitors (e.g. imatinib) in chronic myelogenous leukemia (CML).

Prognostic biomarkers Prognostic biomarkers don't typically trigger specific therapeutic decisions but inform physicians about the risk of clinical outcomes, such as recurrence or disease progression. Examples of prognostic biomarkers include: (i) prostate-specific antigen (PSA) levels which can indicate local and/or systemic prostate cancer disease progression; (ii) chromosome 17p deletions and TP53 mutations which predict poor survival in patients with CML; (iii) BRCA1 and BRCA2 gene mutations that increase the risk of breast and ovarian cancer in women and prostate cancer in men.

Moreover, hypermethylation of a gene promoter as a prognostic factor can lead to loss of gene expression, such as methylation of the HSBP1 gene in prostate cancer. Meanwhile, the presence of circulating tumor cells (CTCs) in peripheral blood which is strongly correlated with metastasis and the formation of secondary tumor foci, is considered as a prognostic factor as well.

Diagnostic Biomarkers are broadly used to identify whether a patient has a specific disease condition and are expected to have high specificity and sensitivity. They include: (i) the Bence-Jones protein urine test which is often used as a diagnostic indicator of multiple myeloma; (ii) elevated levels of the CEA which is used in the surveillance of CRC patients; (iii) levels of PSA which are higher in patients with prostate cancer.

For instance, the identification of EGFR-related mutations in lung cancer, determines the response to EGFR inhibitors, leading to improved treatment efficacy and patient survival rates in prognostic sense. The advent of those biomarkers has revolutionized the field of PPM, where treatments are tailored to individual patients based on their unique disease characteristics. By providing insights into the likelihood of treatment response, predictive and prognostic biomarkers empower clinicians to make informed decisions and optimize therapeutic interventions (13).

A Grand challenge for the development of cancer biomarkers is the nature of the cancer as being a heterogeneous disease. This heterogeneity may complicate the development of biomarkers. Detailed and comprehensive knowledge of cancer at the cellular and molecular levels has grown dramatically and exponentially in the past two decades and has resulted in significant improvement in the characterization of human tumors which in turn has catalyzed a shift toward the development of targeted therapies, the basic concept for PPM. Therefore, it is postulated that the emergence of highly powerful "OMICS" technologies would contribute a lot into the backbone toward the discovery of novel biomarkers and/or panels, with distinct advantages over the currently used biomarkers. And the former concept of single biomarker discovery is expected to be replaced soon by multi-biomarkers discovery of panel of genes or proteins whereby, rising the query of whether the heterogeneous and multifactorial cancer may have single fingerprint.

And thus, expectations regarding the level of precision for such tools will likely be increased by the perception that Big Data and Data Banks (for example, clinical databases, high-throughput experimental datasets, IT-resources) can be translated into clinically relevant and useful information.

So, the joint effort of clinicians, biodesigners, researchers, bioinformaticians, and biostatisticians, in academia and industry will certainly make progress towards the development of sensitive and specific predictive, prognostic and diagnostic biomarkers. And the latter as a Grand Challenge and future prospective of biomarkers would determine trends in the biomarker development by facilitating the combination of therapeutics with diagnostics and would promise to play an important role in the development of PPM.

The intended use of a biomarker (e.g., risk stratification and screening) and the target population to be tested need to be defined early in the development process. The use of a biomarker in relation to the course of a disease and specific clinical contexts should also be pre-specified. The patients, persons-at-risk (pre-cancer clients) and specimens should both directly reflect the target population and intended use. And key considerations for conducting discovery studies using archived specimens are thus the targeted population represented by the specimen archive, power of the study, prevalence of the disease, the analytical validity of the biomarker test, and the pre-planned analysis plan.

With the emergence of more sensitive and specific technologies that are now able to be run in clinical settings and the ability to accurately measure biomarkers, there is a need to understand how biomarkers are defined, and how they are used in conjunction with drug treatment or with the frame of protocols of clinical trials (10-14). And the unique molecular and genomic heterogeneity of the living systems, including humans, constitutes a potentially rich source of candidate biomarkers (9). In this sense, the refinement of a set of candidate biomarkers can be achieved through many different pipelines. But clearly, the identification of better candidate biomarkers at the beginning of the development pipeline will prove beneficial in the later stages of the process.

Innovative clinical trial designs are needed to address the difficulties and issues in the development and validation of biomarker-based personalized therapies. Designing trials of biomarker-guided therapy has many challenges, including:

- (i) being almost always unblinded, they are prone to bias;
- (ii) the control group, most frequently ‘usual care’ group, is open to contamination and has inevitably better outcome than in real non-trial ‘usual care’;
- (iii) being per essence ‘strategy-trials’ rather than simple intervention trials, causality is difficult to establish;
- (iv) therapy optimization as a result of change in the tested biomarker may be left to the decision of the investigator, only instructed to follow best guideline medical therapy, or decided per-protocol using more or less sophisticated algorithms, which, although guideline-based, may vary according to the protocol (10-14).

Identification, qualification and implementation of the different kinds of biomarkers are challenging and frequently necessitate collaborative efforts. But to fully realize the resources of PPM, two components are essential: (i) a **targeted therapy** and (ii) a **companion test** to identify a biomarker (**Fig. 8A, B**).

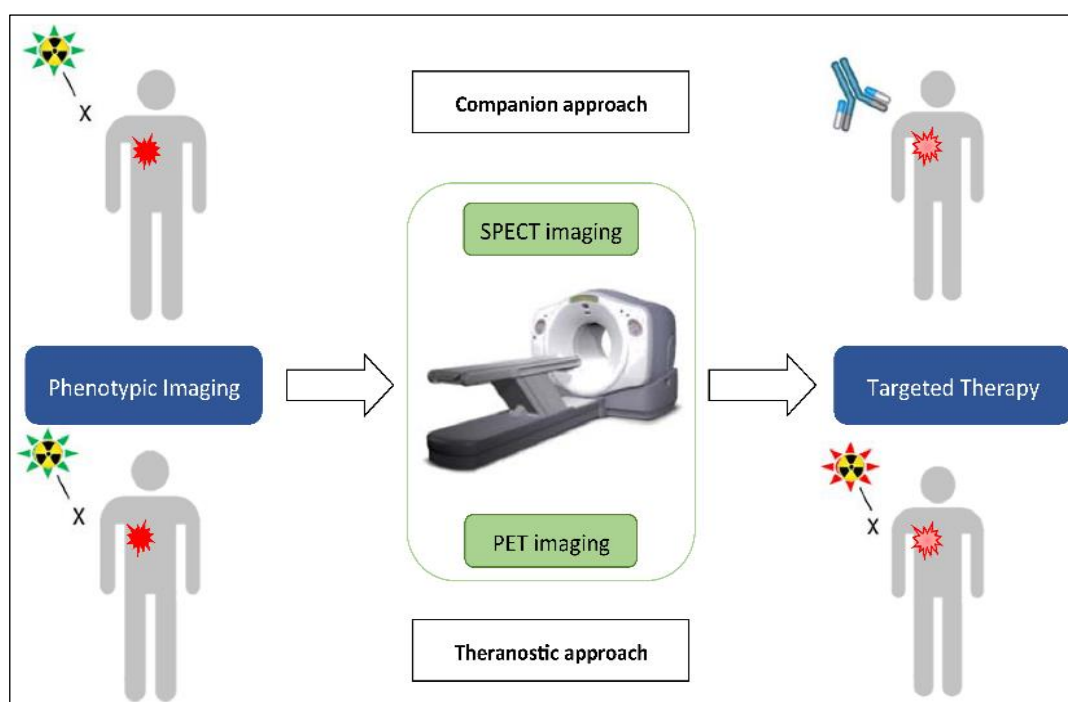


Fig. 8A: Companion and theranostic approaches in PPM-driven nuclear medicine

“Theranostics” is an approach, in which single chemical entities are developed to deliver therapy and diagnosis simultaneously. The advent of theranostics in nuclear medicine has opened new opportunities for the diagnosis and treatment of cancer patients. Getting access to phenotype of metastases would allow better management of patients. In the context of metastasis heterogeneity, nuclear imaging and the companion approach allows phenotyping of secondary lesions, in a non-invasive and very sensitive manner.

Phenotypic imaging (left) achieved using a PET- or SPECT-dedicated radiolabel compound can be followed by a non-radioactive therapy (right, top) or a radioactive therapy (right, bottom)

PET, positron-emission tomography; PPM, personalized & precision medicine; SPECT, single-photon emission computed tomography

The source: Montemagno, C.; Pagès, G. Metastatic Heterogeneity of Breast Cancer: Companion and Theranostic Approach in Nuclear Medicine. *Cancers* 2020, 12, 821. <https://doi.org/10.3390/cancers12040821>

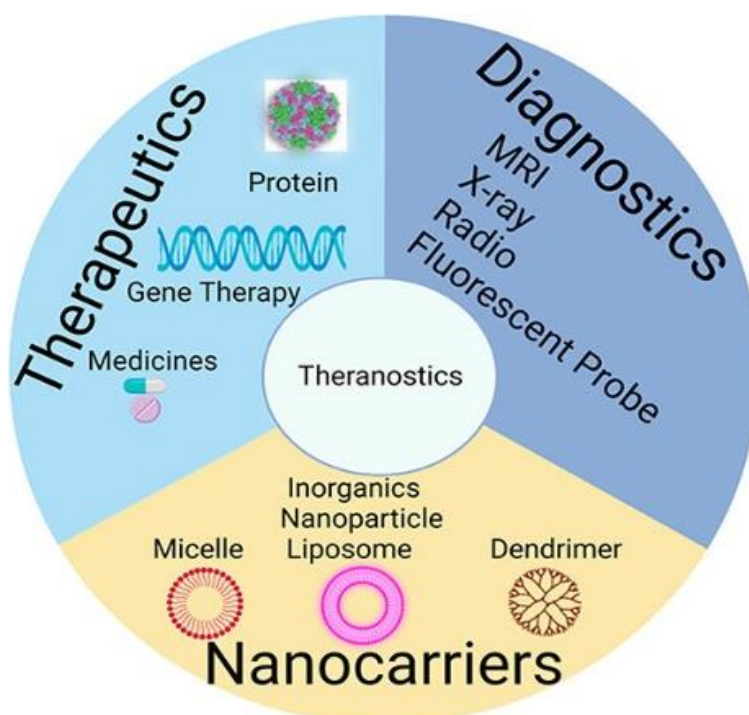


Fig. 8B: Nanotheranostic platform for simultaneous targeted therapy and theranostics-driven diagnosis

Comprehensive smart nanoplateforms from organic, inorganic, and carbon-based nanoparticles for cancer theranostics, which offers an exciting opportunity to gain targeting strategies and multifunctionality. The advent of nanotechnology, in particular the development of smart nanomaterials, has transformed cancer diagnosis and treatment.

CT, computer tomography; MRI, magnetic resonance imaging; PET, positron-emission tomography

The source: Montemagno, C.; Pagès, G. Metastatic Heterogeneity of Breast Cancer: Companion and Theranostic Approach in Nuclear Medicine. *Cancers* 2020, 12, 821. <https://doi.org/10.3390/cancers12040821>

The latter is particularly true for stratification biomarkers that require a **companion diagnostic marker (theranostics)** that is co-developed with a certain drug (**Fig. 9A, B**).

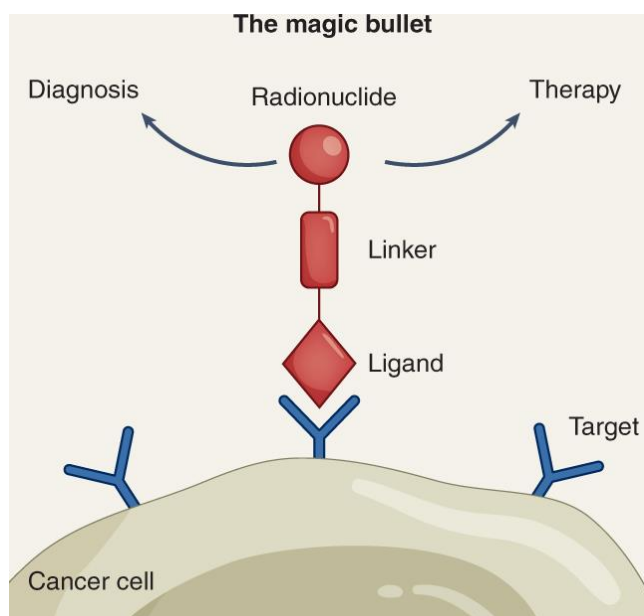


Fig. 9A: Stratification biomarkers via a companion diagnostic marker (theranostics)

Theranostics (companion diagnostic) aims to both diagnose and treat cancer. Although few such drugs are on the market, many are being tested in clinical trials, with early results showing promise

The source: Arnold, C. Theranostics could be big business in precision oncology. *Nat Med* 28, 606–608 (2022). <https://doi.org/10.1038/s41591-022-01759-6>
<https://www.thno.org/v06p1274.htm>

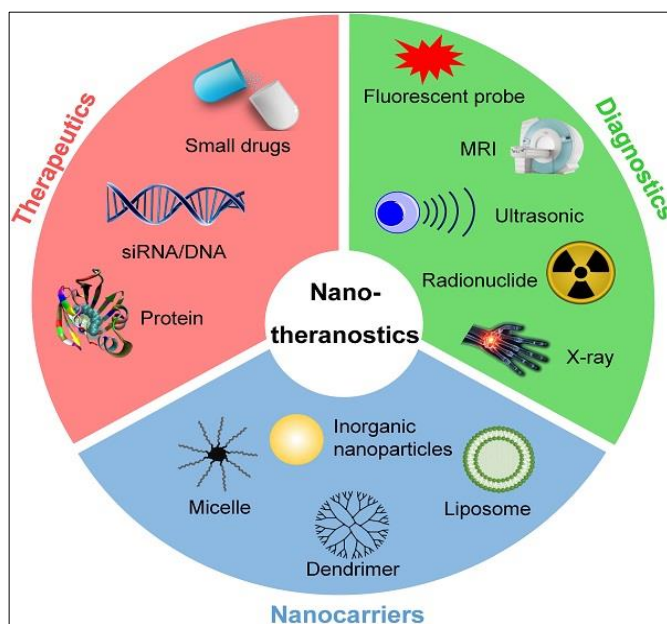


Fig. 9B: Nanostructure-Based Theranostic Systems

Theranostics aims to combine therapeutic and diagnostic tools to create a more efficient treatment option for various diseases. It has been closely tied to PPM in the hopes of creating

protocols tailored to individuals. Nanotheranostics, which integrates both therapeutic and diagnostic capabilities into one single nanoplatform, have drawn wide attention owing to their potential as theranostics for simultaneous therapy of cancer, and has provided exciting prospects for optimizing treatment outcomes in cancer and other diseases.

The source: Wang, J., Cui, H. (2016). Nanostructure-Based Theranostic Systems. *Theranostics*, 6(9), 1274-1276. <https://doi.org/10.7150/thno.16479>

The above-mentioned is considered to be the future of PPM, being and serving as a valuable guidance and playing a crucial role in clinical practice since are possessing their accuracy to be crucial for the success of the therapeutic, preventive, prophylactic and rehabilitative choice. All emerging treatments and associated biomarkers require clinical trials to confirm their properties and to inform and influence daily clinical practices, as well regulatory reporting before achieving approval for professional and/or commercial release. In this context, biomarker-adaptive signature designs (ASD) identify the most suitable target subpopulations, based on clinical observations or known biomarkers, evaluate the effectiveness of the treatment on that subpopulation in a statistically valid manner, and combine the biomarker identification and classifier development to the selection of candidate patients and a statistical test for treatment effect on the selected patient subgroup for clinical trials.

Biomarkers can be used in clinical settings to facilitate drug repurposing and inform patient care decisions, and can be incorporated into drug development through the drug approval process, scientific community consensus followed by regulatory acceptance, and biomarker qualification.

The involvement of biomarkers in clinical practices will be more and more common in the next 5–10 years because of the development in medical-related biological and transdisciplinary research, as well as in biodesign-inspired and biotech-driven translational applications. More clinical questions need to be answered about the biomarker and its role in disease process, and therefore more biomarker-related clinical trials will be designed to answer those specific questions. More flexible trials serving multiple purposes are expected due to the intricate relation between biomarkers and the disease.

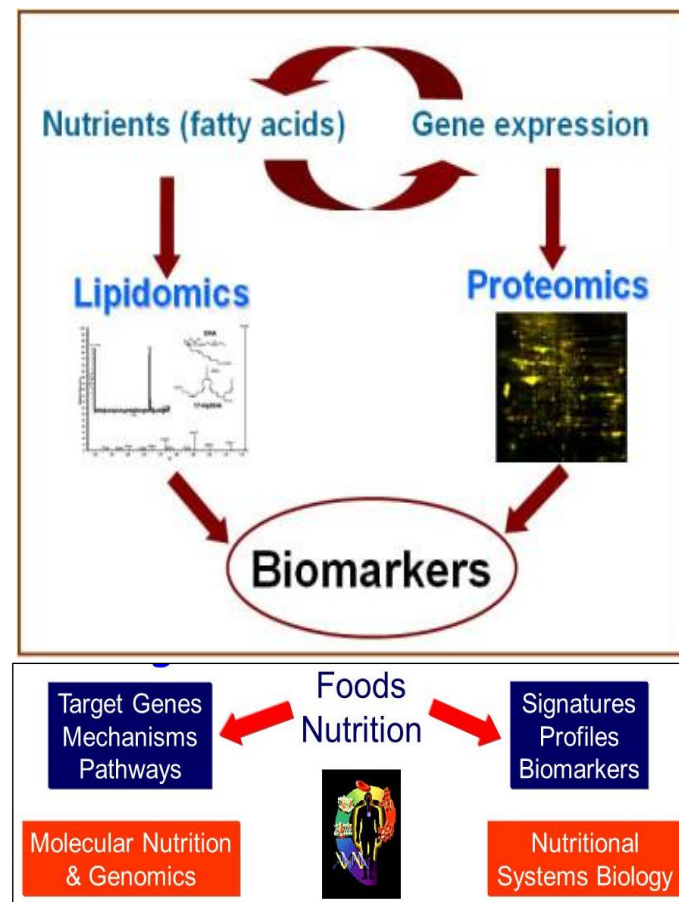


Fig. 10: Nutraceutical biomarkers

Food intake measurement is based on self-reported dietary intake questionnaires, which have inherent limitations. They can be overcome by the use of biomarkers, capable of objectively assessing food consumption without the bias of self-reported dietary assessment. Nutraceutical biomarkers could analyze physiological or pathological responses to certain food components or diets, identify persons with specific dietary deficiency, provide information on inter-individual variations or help to formulate personalized dietary recommendations to achieve optimal health for particular phenotypes, currently referred to as “precision nutrition.”

Meanwhile, in a few areas related to human personalized nutrition and metabolism, biomarkers play important roles to predict health and functional outcome, and are routinely used in clinical practice (**Fig 10**).

Therefore, intervention strategies are mostly still targeted at individualized and population levels and nutri-, epi- and metagenomics biomarkers could play a crucial role in decision making, whilst influenced by dietary, lifestyle, and environmental factors, which contribute to the heterogeneity observed in humans. So, biomarkers of the next step generation which indicate the individual risk or benefit must not neglect the complexity of foods, lifestyle, and metabolic processes that contribute to health or disease and are significant challenges for personalizing dietary advice for healthy or diseased individuals.

Meanwhile, in global terms, a principally new generation of biomarkers is required that define all aspects of the variability of unified system indicators. For instance, circulating microRNAs (miRNAs) are attracting interest in the burgeoning field of PPM and associated subfields, with data supporting their diagnostic, prognostic and predictive biomarker potential (**Fig 11**).

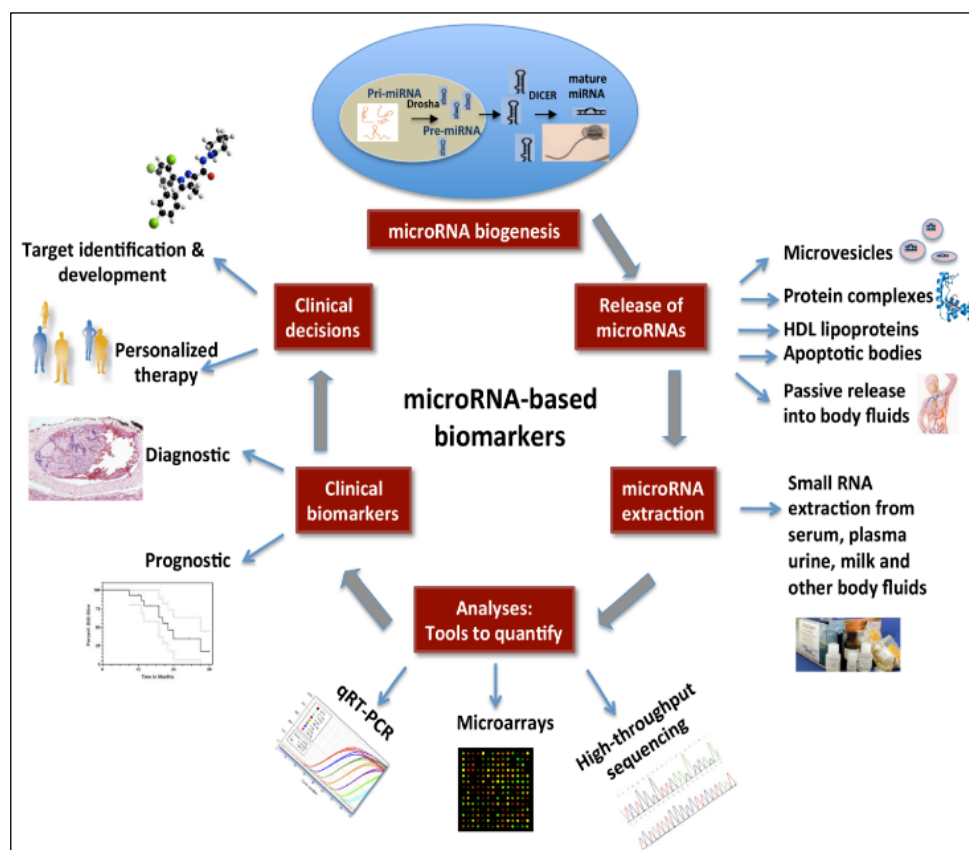


Fig. 11: Circulating microRNAs as biomarkers

A schematic diagram showing miRNA biogenesis, modes of their secretion into body fluids, RNA extraction and quantitative approaches. In future, clinical decisions may be made based on the expression levels of miRNAs

MicroRNAs (miRNAs) are small non-coding RNA molecules, which in recent years have emerged to have enormous potential as biomarkers. miRNAs as biomarkers provide possibilities for development of less or non-invasive and more specific methods for monitoring tumor growth and progression. The prospect of miRNAs as potential diagnostic and prognostic biomarkers with clinical applications is significant as more evidence points to their central role in cancer pathobiology.

Sundarbose, K.; Kartha, R.V.; Subramanian, S. MicroRNAs as Biomarkers in Cancer. *Diagnostics* 2013, 3, 84-104. <https://doi.org/10.3390/diagnostics3010084>

Effective miRNA profiling calls for reproducible, sensitive and specific tools with turn-around times fast enough to support Biodesign-inspired translational research and applications into what can be a rapidly changing disease progression and treatment environment.

Moreover, following the clinical aims and objectives of the next step generation and having a complete understanding of a drug's pathway, interactome, and network interactions could expedite the identification of sensitizing mutations, drug interactions, or the risks of drug combinations to guide biomarker discovery, including *simple*, *combinatorial*, and *network-based* biomarkers (*NBBs*) (Fig. 12A-C).

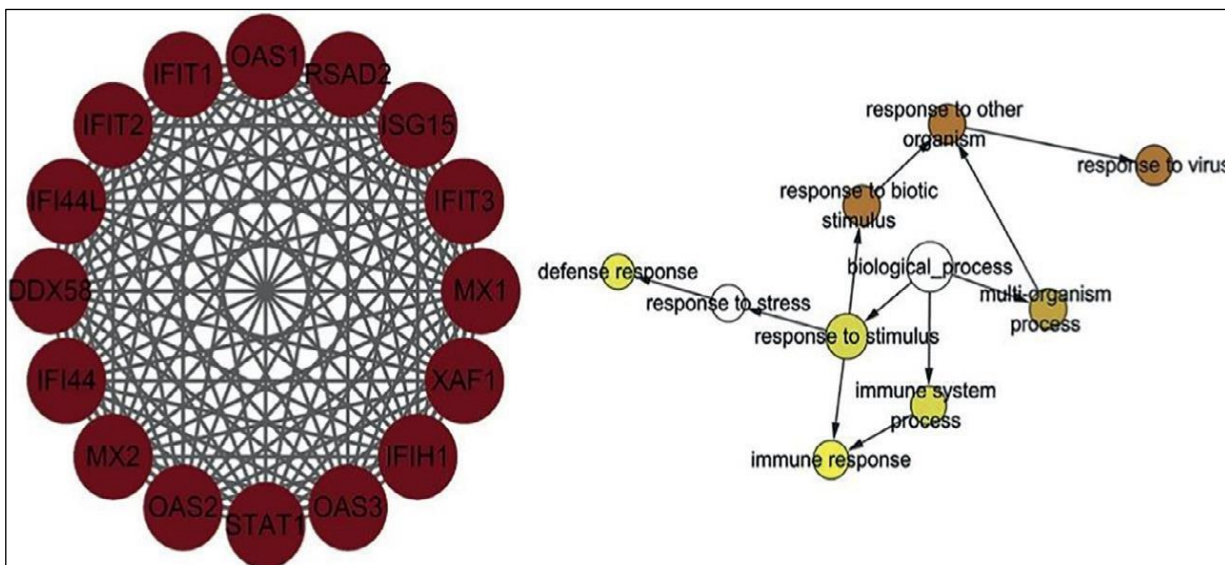


Fig. 12A: Potential NBB-related protein biomarkers for systemic lupus erythematosus (SLE)

determined by bioinformatics analysis

SLE is a heterogeneous autoimmune disorder, featuring with 90 (82 up- and 8 downregulated) differentially expressed genes (DEGs) common to female LN-, female LN+, and male LN+ using the GSE65391 and GSE49454 gene expression datasets from Gene Expression Omnibus database. The protein-protein interaction (PPI) network of 70 DEGs was constructed using STRING and cytoscape, and the Gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis showed that the PPI network was significantly enriched in defense response to virus, cytosol, protein binding, and measles. Sixteen hub genes were identified from this PPI network, and Literature Mining Gene Networks molecular of GenCLiP 2.0 showed strong interaction between STAT1, DDX58, and IFIT1. Enrichment analysis of hub genes in published literature showed the involvement of immune response and interferon-related genes in the pathogenesis of SLE. In addition, the transcription factors STAT1 and 2 and IRF6 and 9 had high Normalized Enrichment Score. The 70 DEGs with PPI network and 16 hub genes are potential biomarkers of SLE, and can help improve diagnosis and develop individualized therapies

NBB, network-based biomarkers; **SLE**, systemic lupus erythematosus; **PPI**, protein-protein interaction

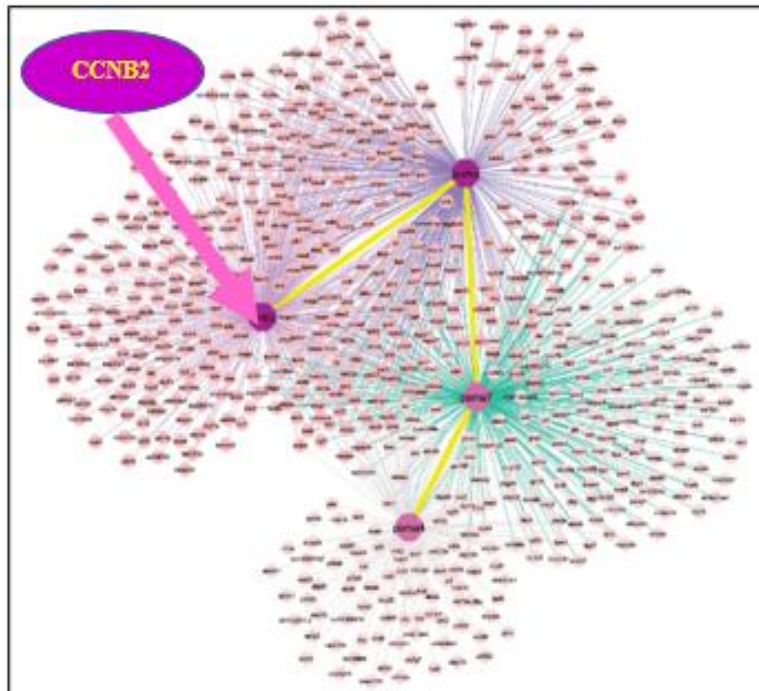


Fig. 12B: CCNB2 as a Potential Non-Invasive Breast Cancer Diagnostic Biomarker in Peripheral Blood Mononuclear Cells Using the interactome approach

Co-expression network of four major hubs in breast cancer tissue; **purple** nodes are hub genes of the network. **Yellow** lines indicate interaction between the hub genes
CCNB2 has a significant role as a prognostic NBB in cancer progression

NBB, network-based biomarker

Source: Moradpoor R, Zali H, Gharebaghian A, Akbari ME, Ajdari S, Salimi M. Identification of CCNB2 as A Potential Non-Invasive Breast Cancer Biomarker in Peripheral Blood Mononuclear Cells Using the Systems Biology Approach. Cell J. 2021 Sep;23(4):406-413. doi: 10.22074/cellj.2021.7053. Epub 2021 Aug 29. PMID: 34455715; PMCID: PMC8405074.

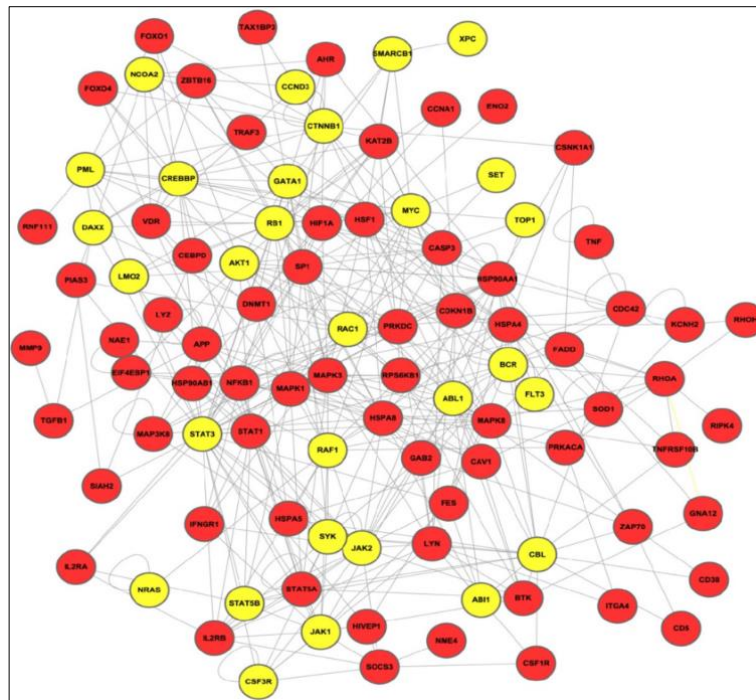


Fig. 12C: The final network-based biomarker for leukemia

The known cancer related genes in final network are marked **yellow**.

Leukemia is highly complex and heterogeneous, involving interaction among multiple molecular components. Network biomarkers are considered to outperform individual molecules in disease characterization. The candidate NBB was evaluated for the diagnosing performance. A network of 97 genes and 400 interactions was identified for accurate diagnosis of leukemia. Functional enrichment analysis revealed that the NBBs were enriched in pathways in cancer. The NBBs provide a useful tool to diagnose leukemia and also aids in further understanding the molecular basis of leukemia.

NBB, network-based biomarker

Source: Xuye Yuan et al. Network Biomarkers Constructed from Gene Expression and Protein-Protein Interaction Data for Accurate Prediction of Leukemia. *J. Cancer*, 2017; 8(2): 278-286. doi: 10.7150/jca.17302

We may also identify specific pathways and interactome-based networks involved in diseases for which drugs have not yet been explored in appropriately designed trials. In this sense, NBBs help determine the probability of developing chronic pathologies or autoimmunity- or cancer-predisposed conditions. Key factors contributing to the growth of the global NBBs-related healthcare services market include high prevalence of chronic autoimmune diseases and cancer; rising adoption of biomarkers for diagnostic, predictive, and prognostic applications; and increasing application in drug discovery and development.

A NBB using constructed protein association networks is a useful tool to highlight the pathways and mechanisms of the lung carcinogenic process and, more importantly, provides potential therapeutic targets to combat cancer. From a systems perspective, the constructed network-

based biomarker further evaluated the targeted carcinogenic process by use of significant protein identification and diagnostic evaluation. More importantly, the significant proteins identified by the NBBs give mechanistic insights into the carcinogenic process and provide potential therapeutic targets to combat cancer in the real clinical practice.

Novel biomarkers may also identify specific pathways involved in risk, where drugs interrupting such mediator bio-targets have not yet been explored in appropriately designed trials (10-12). Regarding biomarkers of the latest innovative trends, let me add that along with canonical antibodies (Abs) serving a crucial role as biomarkers in clinical settings, some of the Ab-based families proven to occur are Abs possessing with catalytic activity (*catAbs* or *abzymes*) and thus to belong to Abs with a feature of *functionality* (Fig 13) (3)

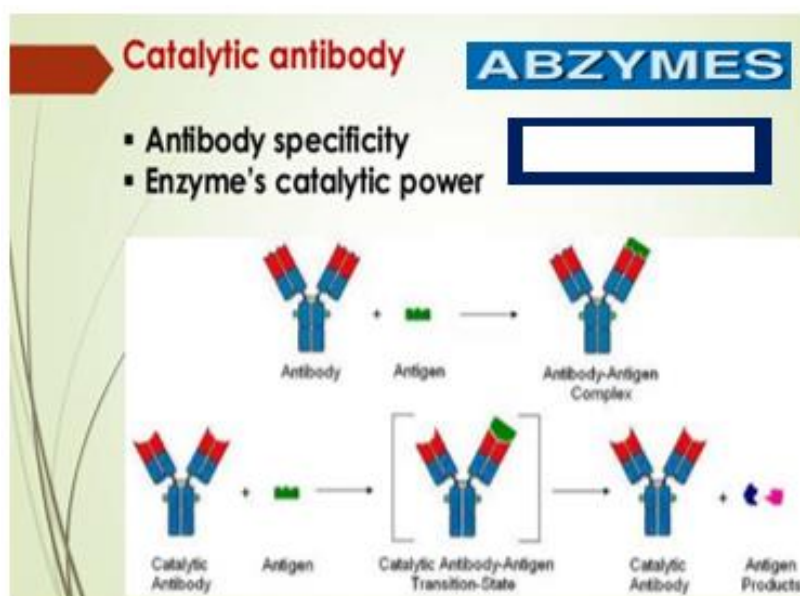


Fig. 13: Antibodies (Abs) possessing with catalytic activity (*catAbs* or *abzymes*) belonging to Abs with a feature of functionality

CatAbs (or abzymes) are multivalent Igs, presumably, of IgG isotype, endowed with a capacity to hydrolyze the Ag substrate. The proteolytic property is buried in the Fab-fragment of the Ig molecule and is appearing to sound as a functional property of the Ab molecule. In this sense, proteolytic Abs (or Ab-proteases) as a significant portion of the big family of abzymes represent Abs endowed with a capacity to provide targeted proteolytic effect

Abs, antibodies; ***catAbs***, catalytic antibodies; ***Ig***, immunoglobulin

CatAbs (or abzymes) are multivalent Igs, presumably of IgG isotype, endowed with a capacity to hydrolyze Ags. The enzymatic activity is located in the Fab fragment of the Ig molecule, which endows such antibodies with the ability to bind to specific antigens and hydrolyze them. Proteolytic Abs (or Ab-proteases) represent a significant portion of the family of abzymes to target specific Ags. Because of their Ag specificity, Ab-proteases also may be used as biomarkers able to control autoimmune disease progression to transform from subclinical into clinical stages, and to predict complications. Moreover, sequence-specific Ab-proteases have proved to

be greatly informative and thus valuable as biomarkers to monitor autoimmune diseases at both subclinical and clinical stages while demonstrating their predictive value for the development of the disorder (3).

You might see from the above-mentioned, that biomarkers can be used along with tools in clinical practice, as drug development tools and can be incorporated into drug development through the drug approval process, scientific community consensus followed by regulatory acceptance, and biomarker qualification. This would offer a new way to optimize treatment and facilitate building new products and services in this area, viz. ***multimarker-based companion diagnostics***. The latter is able distinguishing cancer from normal, benign disease states, and patients with other cancers. The model satisfies the requirements of an ideal screening test, being simple to use, and having a good diagnostic efficacy.

Moreover, the use of a panel of plasma biomarkers for the identification of women with ovarian cancer delivers a significant increase in diagnostic performance when compared to the performance of CA-125 alone (Edgell, T., Martin-Roussety, G., Barker, G. et al. Phase II biomarker trial of a multimarker diagnostic for ovarian cancer. *J Cancer Res Clin Oncol* 136, 1079–1088 (2010). <https://doi.org/10.1007/s00432-009-0755-5>).

The unique diagnostic potential of novel biomarkers and its efficacy correlating with phenotypical expression, would cover neuroinflammation and neurodegeneration, including the applications of biomarker-based strategy in multiple sclerosis (MS), Parkinson and Alzheimer diseases.

A comprehensive understanding of the relevance of each cancer biomarker will be very important not only for diagnosing the disease reliably, but also help in the choice of multiple therapeutic alternatives currently available that is likely to benefit the patients. Cancer biomarkers are broadly categorized into three divisions based on the specific signature it is associated with: diagnostic, predictive and prognostic biomarkers. The therapeutic potential of different biomarkers and their use in clinical trials has also been discussed. Despite the recent advancements, a comprehensive approach on biomarker biogenesis is required to integrate the available information and to translate them as tools of prognostic and diagnostic potential.

However, to many the ultimate potential of biomarkers is to change disease management (***reactive*** mode) into health management (***preventive*** mode), in other words keeping healthy people healthy rather than curing diseased patients. Key drivers for such change are molecular biomarkers that allow earlier and more sensitive detection of onset of disease, better molecular classification of disease, improved personalized treatment, and improved monitoring of treatment effects.

Biomarkers of the future would be used for:

- (i) screening the general population or individuals at risk (panels of screening and predisposition biomarkers);
- (ii) (ii) the detection of the presence of a particular type of cancer (panels of diagnostic and prognostic biomarkers);
- (iii) (iii) monitoring the progression of autoimmune inflammation, and predicting the complications and outcome (panels of prognostic biomarkers);

- (iv) (iv) understanding whether a patient will benefit from a specific drug treatment (panels of predictive biomarkers); and
- (v) (v) evaluating the drug's efficacy and optimizing the treatment, providing the tool to tailor treatment for individual autoimmunity-related patients or persons at risk (panels of pharmacodynamics biomarkers).

Meanwhile, a number of limitations of multimarker-based panels should be acknowledged. These include potential multiplexing and analytical challenges in assaying multiple biomarkers at once as well as the challenges of interpretation for the clinician due to different cut-offs for each of the separate markers (9). Nevertheless, it can be anticipated that scoring calculators and algorithms will increasingly use circulating biomarkers in combination with clinical variables to allow appropriate surveillance and fully informed counselling of the patients, persons-at-risk, their families and other stakeholders in the process of patient care.

Anyway, biomarkers have gained immense clinical value and interest in the practice of PPM and PPM-related subareas. Biomarkers are potentially useful along the whole spectrum of the disease process. During diagnosis, a set of specialized biomarkers can determine staging, grading, and selection of initial therapy. During treatment, they can be used to monitor therapy, select additional therapy, or monitor recurrent diseases and complications. Advances in OMICS-technologies and molecular pathology have generated many candidate biomarkers with potential clinical value. In the future, integration of biomarkers, identified using emerging high-throughput technologies, into PPM-related evidence-based medical practice will be necessary to achieve 'personalization' of treatment and disease prevention.

A growing area of biomarker research in autoimmunity and cancer-related conditions is the search for biomarkers that can predict successful drug-free remission. Stratifying diseases classified according to phenotype is not the only way that biomarkers can be used to forge a molecular taxonomy of disease: they can do so also by breaking down the boundaries of current classifications. That is, biomarkers can be used to uncover molecular similarities between diseases thought to be distinct. This trend is to combine quantitative molecular biomarker data and clinical read-outs to provide a more detailed characterization of a disease state. This enables clinicians not only to diagnose diseases but also to specify the subtype or causal origin of the pathophysiology, potentially leading to a more tailored treatment.

Within pharmaceutical development, biomarkers have matured from being a hype to becoming an intricate part of the decision-making process. Rather than trial-and-error, most if not all drug-development projects have key biomarker read-outs incorporated in their strategy to enable early de-risking of the chosen approach and selection of the best compound moving forward. In parallel and in line with the desire for PPM from society, there is a clear trend with oncology leading the way that new drugs have a companion diagnostic biomarker where possible. Supported by their increased economic power, the emerging countries currently invest heavily in biomarker development, aiming to improve existing therapeutic treatments and to generate more personalized drugs for their markets. This progress has dramatically changed the landscape of biomarker discovery and strongly increased the potential of identifying more specific and applicable biomarkers for PPM.

New strategies assisting in the early and pre-early identification of biomarkers also help to optimize clinical trial design through data informed decisions. Biomarkers identified in subclinical development can be translated into the clinic as companion diagnostics (CDx)-theranostics tandem, to stratify relevant patient populations for treatment. Those biomarker-driven approaches are fueling PPM resources to improve drug efficacy, patient safety, and to reduce the attrition rate of the newest therapeutics and nutraceuticals.

Biomarkers and PPM have introduced a novel way of thought processes, appraising diseases, in applying novel advanced technologies, and emphasizing proactive and preventive medicines. Looking ahead, focused biomarker development networks as outlined above are likely to boost the application of validated biomarkers in biodesign-driven translational and PPM. Biomarkers are providing value across the entire drug development spectrum and the shift is impacting both the patients and the entire landscape of the healthcare system.

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