# Biomarkers in personalized medicine: discovery and delivery

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The rapidly developing field of personalized or precision medicine is moving clinical practice towards a new paradigm centred on 'right patient – right medication – right time'. Such information is obtained through analysis of biomarkers, usually specific proteins or DNA sequences. To expand the range of conditions in which a precision approach can be used, it is vital that new biomarkers continue to be discovered and qualified as having clinical utility. Once this is achieved, it is just as important that clinically validated assays to measure these biomarkers are made available to clinical groups to guide prescribing practice.

## Introduction

It is widely recognized that a seachange is taking place in healthcare. The historical approach of classifying cases of disease within broad categories and following empirical treat-and-observe therapeutic strategies is being replaced by a more targeted form of medical practice, which aims to characterize and treat illnesses on the basis of progressively greater understanding of their underlying molecular pathology. This is known as 'personalized' or 'precision' medicine.

Personalized medicine is highly dependent upon biomarkers, specific analytes that can be used to enhance diagnosis in order to help to predict likely treatment responses and guide treatment choices.

The challenge now faced by the diagnostics and pharmaceutical industries is to maintain and preferably increase the rate at which novel biomarkers can be discovered, qualified as having clinical value, translated into validated assays and, ultimately, introduced into routine use. Unfortunately, this process has historically proven to be challenging.

## The biomarker discovery problem

Development of precision medicine requires a pipeline of novel candidate biomarkers which can improve the quality of care delivered in illnesses that cause significant morbidity and mortality, particularly the chronic conditions which affect large numbers of the aging global population such as cardiovascular and neurodegenerative disorders.

To date, most personalized medicine successes have centred on one particular factor, such as identification of a specific mutation in a signalling protein and development of a selective antagonist for this molecule. In order to broaden the range of conditions to which a stratified approach to healthcare can be applied, we must tackle the challenge of biomarker discovery and development in illnesses which often have complex and currently incompletely understood underlying pathology; this makes the task very difficult.

Many biomarker discovery projects, particularly those conducted in conditions that are considered unlikely to have a single underlying cause such as a specific driver mutation, have employed hypothesis-free '-omics' technologies to search for changes in a wide variety of analytes in the expectation that some will be discovered which are highly characteristic of a particular disease.

Although an appealing concept, this approach comes with its own particular set of complexities and difficulties which must be fully understood and mitigated against if a project is to stand a chance of success.

## Maximizing the chances of success

Discovery, validation and clinical qualification of novel biomarkers using '-omics' approaches is a challenging undertaking. Unfortunately, all too often researchers have carried out studies which, with the benefit of hindsight, were compromised from the outset.

In order to maximize the chances of successfully identifying candidate biomarkers which have true clinical utility, it is important to bear several factors in mind.

*Characterize the performance of the chosen measurement platform*. Carry out *extensive* characterization – when the success of the entire project rests on the analytical values generated by an instrument, it is vital that performance is reliable. Statistical Process Control (SPC) methodologies are particularly helpful here and Biosignatures staff have published two articles[1,2] discussing the application of SPC to research proteomics.

*Standardize all sampling and experimental procedures*. Humans are extremely variable research subjects and, unless the condition investigated has a single characteristic change, the signal-to-noise ratio in samples is likely to be low. Adding technical sample-handling variation to this could severely compromise the ability of the study to generate meaningful results.

*Understand the clinical population of interest*. The population in which a biomarker is intended to be used must be thoroughly understood in order to design an effective discovery study. Candidate biomarkers identified in samples which are not representative of the intended final patient group are much less likely to have the desired high performance in validation and qualification studies.

*Utilize a pragmatic and flexible study design*. There are many factors, such as actual recruitment rate and intra-cohort variability, which have

significant impact on the viability of a study but are difficult to accurately predict *a priori*. Designing studies which incorporate features such as interim assessment points and flexible overall recruitment targets allows adjustments to be made in the light of relevant measurements made on the actual cohort of interest, such as updating statistical power calculations based on the level of inter-sample variation detected.

*Evaluate true assay performance using blind tests*. By designing projects to include both discovery and evaluation phases, it is possible to perform a blind assessment of assay performance immediately after the discovery stage. This allows a more informed decision to be made by the research team regarding project continuation and also demonstrates to third parties that the assay performance figures reported are reliable.

## Translation from R&D to the clinic

Once biomarkers have been discovered and validated in a research context, assays must be produced which are suitable for use in clinical diagnostic laboratories. This generally involves translation from a discovery analytical platform to an automated system, often based on established technologies such as immunoassay, PCR and, increasingly, DNA sequencing. Regulatory approval must also be obtained before assays are placed on the market.

There is an expectation that with greater understanding of diseases and improvements in technology, precision medicine can provide huge potential for clinical benefit to individual patients.

Although the concept of personalized medicine is not new (for example, blood typing before transfusion has been used for many years), the exponential growth in the available information, particularly in genetics or other '-omics', and advances in computational analysis tools, creates an environment in which the opportunity for discovery is high.

The challenge from a clinical perspective is how to implement this new and exciting knowledge. That this is not an easy challenge is reflected in the international efforts to translate academic findings to the clinic.

The US Precision Medicine Initiative will see a US$215 million investment in 2016 that will support efforts in cancer genomics and extend precision medicine to all diseases by creating a national cohort of patients, studying one million Americans to expand the understanding of health and disease.

In the UK, a number of initiatives have been created; members of the UK's Stratified Medicine Programme Coordination Group have together invested more than £200 million in the field over the last 5 years. This has helped to create the infrastructure and access to academic and clinical expertise required to improve patient outcomes. The Precision Medicine Catapult aims to make the UK the most compelling location in the world for the development and delivery of new targeted approaches. And the 100,000 Genomes project, currently the largest national sequencing project of its kind in the world, will sequence 100,000 genomes from patients with rare disease and cancer by 2017. The resulting combination of genetic information and clinical data will enable clinical, academic and industry collaborations to better understand disease and develop new treatments for the benefit of the NHS and its patients.

## Implementation is key

The time, effort and cost involved in biomarker discovery and assay development is not insignificant. But without understanding the clinical care pathways and needs of the medical and laboratory teams involved, promoting adoption of the assay may be challenging. Key barriers must be considered.

*The biomarker must provide treatment or prognostic information*. Demonstrating the need for your assay is paramount. For example, is the information that it will provide actionable? Will it add information not available to the clinician from any other source? If it is a companion diagnostic to a targeted therapy, is the approval and funding stream for the use of that drug secure?

*Integration with the existing care pathway*. Adoption of a new assay is much more likely if it can integrate into existing processes without significant impact such as delay to treatment. As an example, a sequencing assay that may provide substantial genetic information on antibiotic resistance but takes 3 days to complete will not replace a microbiology assay that may give less precise, but still useful, information, in a timeframe more appropriate to begin treatment quickly.

*Availability of appropriate sample material*. Pathology samples in particular can be precious and rare, and there is often high demand for the sample for existing tests. Assays that are sensitive enough to use low amounts of input material are important.

*Increased functionality*. There are obvious advantages to assays based on well-understood and familiar techniques that do not require capital investment in the laboratory. One platform, one assay and one target tests can be expensive as well as using significant amounts of sample material, and introduce unnecessary time delays.

*Clinically relevant turnaround time*. Assays must be scalable to the volumes appropriate for the environment in which they are going to be delivered, be that point of care or centralized laboratory testing. They must be able to provide a clinically relevant turnaround time and sometimes faster is not always better. For example, if the results of one test need to be taken in the context of a range of other tests, having that result sooner may not be an advantage.

*Ease of interpretation of results*. The interpretation of clinically relevant information can be challenging, particularly in complex diseases. However, clear definition of a result and what it means is important.

## Concluding thoughts

Although biomarker research is undoubtedly challenging, trying to understand the nature of the problems it presents more completely and thereby designing and carrying out the best studies that we are able to will maximize the number of projects that successfully achieve their aims. Ultimately, this will lead to the development of better targeted therapies and deliver precision medicine to finally bring about individualized patient management in a wider range of conditions. ■

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# MicroRNAs and their role in personalized medicine

## Using miRNA qPCR panels as a tool to understand cancer

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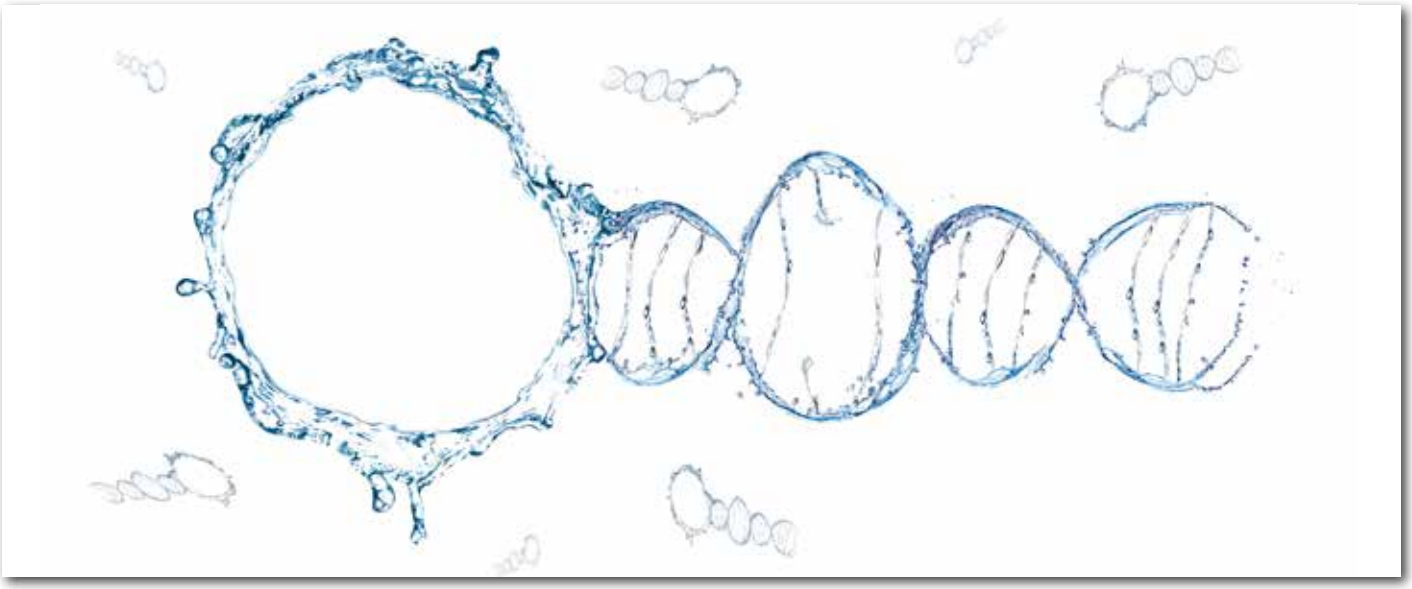
Circulating microRNAs (miRNAs) are attracting interest in the burgeoning field of personalized medicine, with data supporting their diagnostic, prognostic and predictive biomarker potential. Effective miRNA profiling calls for reproducible, sensitive and specific tools with turn-around times fast enough to support investigations into what can be a rapidly changing disease progression and treatment environment. Bioline specializes in miRNA reverse transcription (RT)–quantitative PCR (qPCR) technology and has recently developed a cancer-screening miRNA panel containing 352 targets that uses a DNA-binding dye called SYBR® Green for detection. This panel has the potential to help individualize cancer diagnostics and treatment.

### miRNAs in cellular processes and disease

Since their discovery a little over 20 years ago[1], miRNAs, previously overlooked within what was thought to be non-functional genome components, are now understood to be crucial regulators of important cellular functions[2]. The biogenesis of a miRNA follows a complex path through a number of precursor forms resulting in the mature single-stranded miRNA which is ~20–25 nucleotides (nt) in length[3]. A mature miRNA interacts with mRNA effecting post-translational gene regulation of cellular processes such as development, differentiation, proliferation, metabolism and apoptosis[2], thus it is no surprise that aberrantly expressed miRNAs are a hallmark of many diseases, including cancer[4]. In 2010, miRNAs were included within the traditional oncogene definition due to their vital role in controlling cell differentiation, proliferation and survival[3]. Furthermore, their role in the negative regulation of tumour-suppressor genes is clearly evident[4], and now over 12,600 publications are listed in the NCBI PubMed database relating miRNAs to cancer[5].

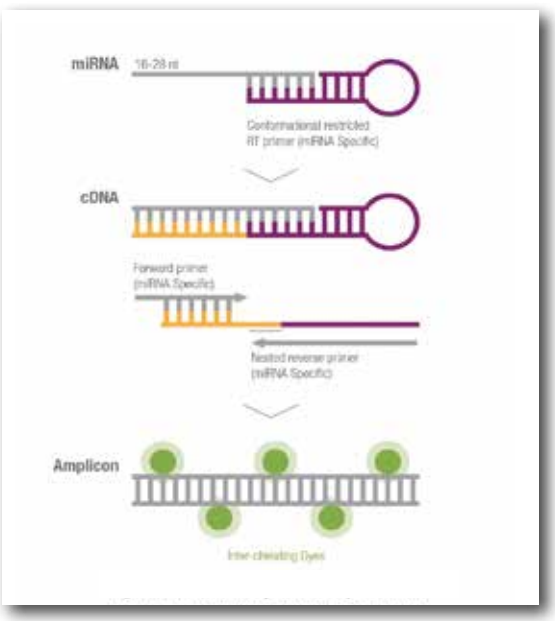
Improvements in deep sequencing technology have allowed for genome-wide profiling of miRNA expression, revealing cancer-specific signatures that not only discriminate between cancer types with high accuracy, but also identify tissues of origin in metastasized cancers[4], thus miRNA profiling has become an attractive concept for development as a cancer diagnostic. Furthermore, the highly regulated process of miRNA expression is sensitive to internal and external stimuli such as hormones and pharmacological molecules, leading to a unique miRNA profile within different tissue types, locations and time points, making them ideal candidates for prognostic and therapeutic oncology biomarkers[5].



## Liquid biopsies and miRNA profiling

There is much excitement around the concept of 'liquid biopsy' – the ability to screen, monitor and uniquely characterize tumours from, for example, a simple blood or plasma sample, foregoing the traditionally invasive, costly and, in many cases, difficult to obtain tissue biopsy[6]. Although circulating cell-free tumour DNA (ctDNA) and circulating tumour cells (CTCs) are commonly the focus for these methods, circulating miRNAs are also attracting attention as viable candidates. Not only do they carry specific information about the pathophysiological state of an individual, but also miRNAs are remarkably stable in their protein-bound form and are present in cell-free body fluids such as plasma, serum, urine and saliva[7]. Correlations have been observed between specific circulating miRNAs and chemotherapy responses in a range of cancers[5], and retrospective studies have also begun to identify miRNA signatures with strong predictive and prognostic potential[8].

There are still many hurdles to overcome before miRNA profiling and liquid biopsy become mainstream diagnostic practice, not least of which is establishing robust and reproducible protocols, in addition to identifying, among the growing list of candidates, which combination of targets can be linked to clinical relevance. Indeed, the very specificity with which cells express miRNAs and the subsequent sensitivity to a range of stimuli, including age and gender, although attractive qualities for personalized medicine, make for a difficult moving target for applied research[5].




EPIK™ miRNA panel assay workflow

## Molecular tools for miRNA profiling

There are currently 2661 known human miRNAs, of which 2556 have been qualified according to the most recent version of the miRBase database. Expression profile studies based on microarray platforms or large-scale deep sequencing projects have been instrumental in the discovery and identification of miRNAs, serving to expand the current database to almost double the number of known human miRNAs over the last 5 years. Among the growing number of tools available for studying miRNAs, however, qPCR remains a routine favourite as it has potential to be extremely sensitive and accurate, as well as being accessible to many in terms of access to instrumentation as well as overall cost. Notwithstanding the range of sample collection and extraction methods with all associated caveats, there are many challenges to overcome when applying RT–qPCR to miRNAs. Targets are short, 18–22 nucleotides (nt), as well as being highly homologous, often with as little as 1–2 nt differences; in addition, the mature miRNA target sequence is present in all the precursor forms[9]. Bioline's EPIK™ miRNA Panels overcome these challenges and the unique assay design discriminates between even the most closely related miRNA targets, such as those within the *let-7* family, with maximum sensitivity down to 10 pg of total RNA, enabling a low volume of starting material, such as required for blood and plasma samples and other liquid biopsy candidates[10].

### What makes a good biomarker?

Biomarkers are characteristics of a sample that can be measured objectively without reference to the clinical symptoms a set of patients are presenting. Ideally the sample will be easy to obtain from the patient - and in most cases this means urine or a small (<15 ml) blood sample.

The biomarker and the method used for analysis should:

- not place the patient at an increased risk
- allow cost savings to be made compared to any existing method
- allow monitoring of the progression of disease
- perform consistently in clinical trials against gender and racial groups

In terms of the biochemistry and molecular biology of biomarker identification, the test should be simple to perform and interpret. In the case of miRNA, the reduction in complexity from analysing the entire transcriptome associated with a disease to only the regulators that are affected by the disease state provides the simplicity in the test. Coupling measurement to a qPCR-based system allows changes in miRNA quantity to be measured as disease state alters. Samples of miRNA can be easily isolated from human circulating fluids so the miRNA panel approach provides a coherent means for biomarker identification.

## Advanced design concepts for miRNA qPCR panels

There are many ways to overcome the difficulties of miRNA priming for RT–qPCR, many of which involve universal priming steps and additions of long-tail nucleotides, resulting in a reduction of qPCR efficiency and target specificity[9]. EPIK™ miRNA Panel assays forego the universal priming approach and incorporate a unique three-primer system, all of which are miRNA-specific, including the initial RT stem–loop primer, allowing for discrimination of mature miRNAs as well as high specificity to identify very closely related targets[10].

Despite the use of specific primer sets, the overall protocol allows interpretation of profile changes in less than half a day, making use of the speed advantage that qPCR holds over sequencing or microarray approaches. To focus study efforts on the most differentially expressed miRNA candidates, when the panel was designed, thousands of miRNA and cancer related articles were screened and only the top 340 targets were used. Additionally, assay performance is enhanced to ensure good-quality quantification, covering targets with low and high expression within a single run[10]. The RT step is linked to SYBR® Green dye detection, negating the use of sequence-specific probes while maintaining specificity with the three specific primer approach.

As efforts continue towards the transition of miRNA profiling from bench to bedside, RT–qPCR panels support current applied research and the resulting clinical processes, covering the most useful targets to monitor disease states, and offering the performance and sensitivity required for low-level starting materials or liquid biopsy applications. ■

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