



# An introduction to Cancer Biomarkers in NSCLC

– methods and platforms, clinical data and future aspects

Authors: Fred R. Hirsch, Odd Terje Brustugun, Åslaug Helland, Janne Lehtiö and Rolf Lewensohn  
 Scientific editor: Fred R. Hirsch



## 2. Technologies used in genomics

### 2.1 DNA Sequencing

DNA sequencing is the process of determining the nucleotide order of a given DNA fragment. This is most DNA sequencing has been performed using the chain-termination method developed by Sanger (Figure 1). DNA fragments of different lengths as a result of chain-termination that is incorporation of a dideoxynucleotide (ddATP, ddGTP, ddCTP, or ddTTP) in four separate sequencing reactions, are separated by gel electrophoresis in four individual lanes (A, C, G, T). The relative positions of the different bands among the four lanes are then used to read from bottom to top the DNA sequence as indicated.

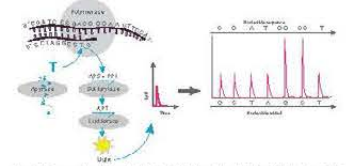
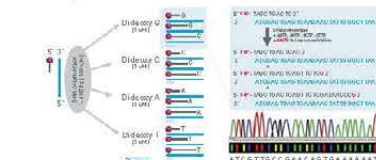


Figure 1.1. Deep sequencing method involves DNA fragmentation and a high number of DNA fragments. DNA fragments are ligated to sequencing adapters. The sequencing process involves PCR amplification of the DNA fragments. The PCR products are then sequenced on a flow cell. The sequencing process involves the incorporation of fluorescently labeled dNTPs. The resulting sequencing data is then analyzed to determine the DNA sequence.

### 2.2 Comparative Genomic Hybridization Analysis

In situ hybridization of differentially labeled tumour DNA against human metaphase spreads (Figure 2). In array-CGH a grid of spotted DNA fragments (e.g. BACs, cDNAs, oligonucleotides) is hybridized to metaphase chromosomes. As the hybridization of the fluorescent probes to the tumour DNA and the normal DNA are compared, gains and losses of DNA are detected. Array-CGH is used for a genome-wide analysis of copy number changes, such as amplifications and deletions (4). This technology can be used to identify copy number changes in the genome. It is used to identify copy number changes in the genome. It is used to identify copy number changes in the genome.

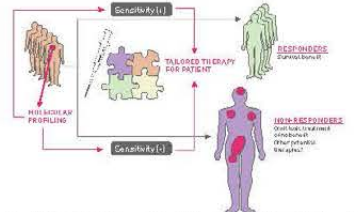
it binds to RNA induced silencing complex (RISC) proteins. The miRNA-RISC complex can bind to complementary sequences of mRNA and inhibit mRNA to be translated to protein.

Several research groups have provided evidence that miRNAs may act as key regulators of processes as diverse as cell development, cell proliferation, cell death, apoptosis and cell metabolism and cell differentiation.

The miRNA array methodology is basically the same as gene expression array technology. A summary table of methods used in genomics can be seen in Table 1.

Method	Application	Advantages	Disadvantages	Resolution	Throughput	Cost
SNP Sequencing	Genotyping, association studies	High resolution, high accuracy	Low throughput, high cost	High	Low	High
CGP	Copy number variation, loss of heterozygosity	High resolution, high accuracy	Low throughput, high cost	High	Low	High
FISH	Gene amplification, chromosome rearrangements	High resolution, high accuracy	Low throughput, high cost	High	Low	High

## The Future – Tailored Therapy



We will in this chapter outline some of the potential markers that we believe will be of importance in future core and clinical studies, both as outcome measures and treatment decisions. We will focus on non-metastatic lung cancer, with this information will also be relevant for other chest malignancies, as most cell line cancers are more similar, and even for most cancer types.

Biomarkers will involve factors related to the tumour itself, the host's constitution (susceptibility of disease) and its response to the disease (Figure 3). These markers will be discussed in more detail in the following sections.

In addition to proteins and genes, a range of imaging modalities, from the conventional X-ray, via functional MRI and PET/CT to novel – perhaps, nanotechnology-based – techniques are increasingly important biomarkers in lung cancer management (see section 4.2).



Figure 3. Biomarkers in the individualized treatment of lung cancer.

### 1.1 Background

Cancer is a genetic disease at the molecular level, a result of several genomic and/or epigenomic alterations accumulated within tumours. In addition, it is increasingly accepted that the host (patient's) genotype and the tumour's surroundings influences the course of a cancer disease and the response to treatment (both radiotherapy and chemotherapy).

Tumour characteristics	Patient characteristics
DNA copy number	DNA methylation (DNMT)
DNA mutation, rearing	Gene expression, RNA profiling
Gene expression, RNA profiling	Serum/plasma microproteins
Serum/plasma microproteins	Pharmacogenetics
Cellular heterogeneity	
Functional imaging	

Figure 1. Biomarkers, either tumour or host related.

During the past years, tumours have been extensively characterized, and we know more than ever about cancer at the molecular level. This has led to the development of changing targeting paradigms in a more 'tailored therapy'. In addition, we have experienced an intensive technological development, particularly enabling us to apply our knowledge about molecular processes in clinical practice.

However, there has been less progress in the verification of biomarkers and the complex correlation between the tumour and the host. Such verification is essential before clinical application. The incorporation of biomarkers in clinical trials is therefore a prerequisite before implementation in clinical practice.

### 1.2 Clinical studies

Virtually all steps in clinical studies could potentially include evaluation of biomarkers (Figure 4). This should also be taken into account in the design phase, as well as selection of relevant biomarkers to be applied throughout the study will lead to a better-tailored treatment phase with power to give additional information about the patient. Also, adverse events could be better understood if these factors are taken seriously from the early planning phase.



Figure 4. Steps in clinical studies including biomarkers.

We find it rational to divide the potential markers in two groups depending on their relation to either the tumour per se, or to the patient as a whole. Clearly, both types of markers may be evaluated at each of the steps mentioned above.

Additionally, imaging as a biomarker is discussed in a separate paragraph but this modality should undoubtedly also be employed at all trial steps.