# DIAGNOSTICS OF HEREDITARY MALIGNANCIES

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*Summary:* Significant advances have occurred in our understanding of the cancer etiology in the last decade, as a consequence of the generalized use of molecular biology techniques in human genetics. Cancer is a form of a complex genetic disease. Most forms of cancer are characterised by the accumulation of different genetic alterations affecting genes from a set of genes with pathogenic potential, which is specific for each tumour entity. While in the majority of malignant tumours these changes are somatically acquired, some mutations are transmitted through the germline and account for an inherited tumour predisposition. The next frontier in cancer genetics is to find genes with high prevalence alleles conferring a low increase or decrease of cancer risk.

*Key words:* diagnostics, cancer, hereditary malignancies

## Introduction

It has already been demonstrated that genomics have indeed begun to change the practice of medicine. If the genomic era can be said to have a precise birth date, it was on April 14, 2003. That was when the international effort known as the Human Genome Project put a close to the pre-genomic era with its announcement (available at http://www.genome.gov) that it had achieved the last of the project's original goals, the complete sequencing of the human genome. The extent and pace of progress in genomics are suggested by the fact that this achievement occurred 11 days before the 50th anniversary of the publication of Watson and Crick's description of the DNA double helix. If science, technology, and medicine have consistently demonstrated anything, it is that they proceed at an ever-quickening pace. That we have gone in the past 50 years from the first description of the structure of our DNA to its complete sequencing gives some indication of how much the impact of genomic medicine on the health care of today's neonates will increase by the time they turn 50 years of age (1, 2).

The discovery of inherited mutations of genes associated with increased risk of cancer has opened a new field of cancer medicine. As these genes have been identified and characterized over the past decade, cancer genetics has become intrinsic to the cancer risk assessment that is an essential component of the practice of preventive oncology. Syndromes have generally been identified based on observation by clinicians. Once the underlying genes have been identified, further research must define the full spectrum of the syndrome, including the gene penetrance (associated cancer risk), identification of any distinguishing histologic, immunohistochemical, or molecular features of the component tumours, details of other associated findings and, ultimately, effective strategies for surveillance and prevention. This process can take many years. The resulting information is essential for individuals and families as they consider whether or when to undergo genetic testing, and the implications of the test result for mutation carriers. Recent guidelines have also emphasized that, in order to competently offer a genetic test to a patient or family, the provider must be prepared to deal with a spectrum of medical, psychological, and social consequences of a positive, negative, or ambiguous result. Some courts have already demonstrated their belief that health care providers bear responsibility for informing patients that their cancer may have an inherited basis, with specific implications for their children (1).

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Table I The genes involved in the predisposition to hereditary malignant syndromes (8)

| Syndrome | Gene | Chromosomal localisation | Localisation/tumour type |
|---|---|---|---|
| MEN2 | RET | 10q11 | Medullar thyroid, pheochromocytoma |
| Hereditary breast/ovarian cancer | BRCA1 | 17q21 | Breast, ovaries, colon, prostate |
| Hereditary breast cancer | BRCA2 | 13q12 | Breast, male breast cancer |
| Hereditary melanoma | CDKN2 | 9p21 | Melanoma, pancreas |
| Hereditary colon polyposis | APC | 5q21 | Intestinal polyps, colon cancer |
| Hereditary retinoblastoma | RB | 13q14 | Retinoblastoma, osteosarcoma |
| Hereditary Wilms tumours | WT1 | 11q13 | Wilms tumour, aniridia, genitourinary abnormalities, mental retardation |
| Li-Fraumeni syndrome | p53 | 17p13 | Sarcoma, breast cancer |
| Neurofibromatosis | NF1 | 17q11.2 | Neurofibroma, neurofibrosarcoma |
| Tuberose sklerosis | TSC2 | 16p13.3 | Angiofibroma, renal angiomyolypoma |
| Von Hippel-Lindau | VHL | 3p25-26 | Kidney cancer, pheochromocitoma |

This review is intended to provide a brief summary of the major inherited cancer syndromes, covering recommendations for genetic diagnostics. The data emphasize some of the most important observations in the last two decades, during which many cancer susceptibility genes have been identified (*Table I*) (3). Epidemiologic studies have elucidated the underlying genetic heterogeneity of even rare tumours: that is, susceptibility to specific tumours may be attributable to mutations in different genes. Conversely, some syndromes that appeared to be independent have been found to be manifestations of different mutations in the same gene. Alternate classifications are surely possible based on gene function, specific tumours, and other factors. The increasing ability to identify individuals at remarkable risk for particular cancers, generally at early ages, has brought with it the responsibility to devise effective surveillance and prevention strategies for these individuals. It is important, therefore, that cancer genetics be included under the aegis of cancer prevention as an area that has become an essential component of medical oncology.

## Cancer as a genetic disease

Cancer can be considered a genetic disease for it is caused by alterations affecting the DNA of somatic cells. However, the definition of cancer as a hereditary disease implies that mutations are already present in the germline and transmitted into families. Pedigree analysis identifies the familial forms of cancer, which are rarer than sporadic forms and are described for almost every type of tumour. These forms may segregate into families as simple Mendelian disorders, but a less penetrant predisposition to develop cancer may also be transmitted as a complex genetic trait (4).

Numerous studies have demonstrated that both somatic genetic changes and hereditary factors are involved in the etiology of many cancers (4, 5). The familial forms are very helpful for the discovery of the genes that increase the susceptibility to cancer and may elucidate on the contribution of a single gene to the disease predisposition. Since very often two or more genetic loci with variable contribution from environmental factors are implicated in tumour predisposition, cancer falls within the category of multifactorial diseases (5).

Polygenic determination may involve: (a) a small number of loci (oligogenic); (b) many loci, each locus having only a small effect (polygenic); (c) a single major locus with a multifactorial background.

Two theories on inherited predisposition to cancer are to be taken into account in order to explain how cancer may be genetically determined. Moreover, genetic heterogeneity is to be considered, in that it may underlie an apparent polygenic determination.

### The two-hit hypothesis

In 1971 a predictive study on retinoblastoma (RB) by Knudson (6) hypothesized that two successive mutations ('hits') were required to turn a normal cell into a tumour cell. In familial RB the first hit is an inherited mutation occurring in the germline that gives tumour susceptibility, and the second is a somatic mutation occurring in the target tissue (retina), which promotes tumour formation. According to this model, sporadic tumours arise from two successive mutations occurring in the same somatic cell. This theory also explains why hereditary cases are more likely to be bilateral and diagnosed earlier. Successively, it was suggested that this model could be applied to other types of hereditary cancer. The identification of the RB gene proved the inactivation of recessive genes as tumour suppressor genes (TSG) via a two-hit mechanism (7).

### The polygenic model

The polygenic theory was postulated to describe the quantitative traits governed by the simultaneous action of many loci as polygenic in Mendelian terms. Since many families show diseases and malformations which cannot be defined as Mendelian traits, the polygenic theory was extended to the so-called discontinuous characters by postulating a continuously variable susceptibility following a Gaussian distribution in the population and the existence of a threshold superimposed on the developmental process. Affected people inherit an unfortunate combination of high-susceptibility genes and their relatives may have a raised susceptibility diverging from the population mean at rate of shared gene proportion. A polygenic inheritance of predisposition to cancer is demonstrated in experimental animals and suggested in humans for different tumours. The polygenic model of cancer predisposition explains the low penetrance through the allele assortment of multiple genes, each having two alleles: 'r' for resistance to cancer and 's' for susceptibility, where 's' is dominant over 'r'. For example, if three genes control tumour predisposition, the risk of cancer would be high in individuals carrying the three 's' alleles, intermediate in individuals with two 's' alleles and low in those with one 's' allele (8).

### Genetic heterogeneity

A disease may also appear genetically complex because of genetic heterogeneity. Genetic heterogeneity is defined as the occurrence of independent mutations at the same locus (allelic heterogeneity) or at more different loci (locus heterogeneity), all causing the same disease phenotype. In case of genetic heterogeneity for a given trait linkage of the disease to markers located in a given chromosomal region will be found in some families, but not in others with the same disease (4).

## General aspects of hereditary cancer risk assessment

### Identifying individuals at increased risk

Dramatic advances in our understanding of the genetic basis of cancer have led to new forms of technology and new tools for assessing the genetic risk of cancer. Although inherited forms of cancer are rare – representing only about 5 percent of many types of adult onset cancer – the risks conferred by the inherited cancer-susceptibility genes are high and the cancers frequently appear at a young age. A common perception of familial cancer is that it is a matter not of whether cancer will develop but of when. Yet since most hereditary cancer syndromes are autosomal dominant, the laws of Mendelian genetics dictate that there is only a 50% chance of inheriting the familial predisposition to cancer. This is where genetic testing can help. When informative, it presents an unprecedented opportunity to prevent the development of cancer (*Table II*) (9).

The majority of patients who develop cancer do so sporadically, that is, there is no familial or hereditary risk. The small percentage of patients with a hereditary cancer syndrome may be suspected on the

Table II  Identifying and testing for hereditary susceptibility to common cancers (9)

| |
|---|
| 1. Pedigree construction |
| 2. Genetic counselling and testing |
| 3. Components of informed consent |
| A description of the purpose and type of test being performed |
| Technical accuracy of the test |
| Implications of a positive and negative result and possibility that the test will not be informative |
| Options for risk estimation without genetic testing |
| Risk of passing mutation to children |
| Fees involved in testing and counselling |
| Psychological implications of test results |
| Risks of insurance and employer discrimination |
| Confidentiality issues |
| Importance of sharing genetic test results with at-risk relatives so that they may benefit from this information |
| 4. Result reporting and post-test counselling |

basis of personal and family medical history. Unfortunately, data gathering around family history is often incomplete. When a family history of cancer is obtained, critical details needed for risk assessment, such as the cancer site and age of diagnosis, are often lacking. This occurs in both primary care and specialty settings. One study showed that the age of diagnosis was documented in only 7% of relatives identified as having cancer. Tools to help facilitate family history gathering, such as medical intake questionnaires, have been in use for many years, but data showing their effectiveness are lacking.

Hereditary cancer syndromes are estimated to account for 5% of diagnosed breast, ovarian, and colon cancers. Although uncommon, they are important to recognize because they confer a high risk of multiple primary cancers occurring at younger ages, affecting multiple members of a family who inherit the cancer-predisposing genetic mutation. More importantly, patients and providers are recognizing the potential therapeutic advantages of identifying hereditary cancer risk. With a growing number of preventive care options available to patients and families with hereditary cancer syndromes, the process of systematically assessing risk is becoming increasingly important. Both provider and patient forces are contributing to the development of hereditary cancer risk assessment. Patients are becoming increasingly aware of cancer genetics through media exposure and, more recently, through direct advertising to consumers of commercially available testing. Patient interest and awareness of testing have in turn affected delivery of genetic services. In fact, patient inquiry about cancer genetic testing is one of the strongest predictors of providers ordering or referring for testing.

### Identification of the cancer susceptibility gene(s) through positional cloning

As shown in numerous studies, there is a high likelihood that cancer has a familial component (10, 11). There are two main ways in which such susceptibility genes may be identified. The first is the identification of a biological or biochemical anomaly associated with the disease – due to the identification of the biochemical change, the causative gene may then be identified. The second technique is reverse genetics, which allows the identification of the causative gene without prior knowledge of its biological function. Therefore, as the majority of genetic diseases identified cannot be associated with an obvious biochemical change, reverse genetics has been the technique of choice for identification of susceptibility genes. Reverse genetics uses the fact that the disease in question is familial, and hence members of an affected family will share a common inherited factor that is causing the disease. As reverse genetics is reliant on this fact, the first step is to identify that the trait of interest is a genetic trait.

Some inherited traits can be the result of somatic translocations of chromosomes which are segregated through the affected family, for example, the *FHIT:TRC8* translocation which was shown to cause susceptibility to renal cancer, and, to a lesser extent, PTC (papillary thyroid carcinoma) (12), and, as stated above, 5q21 was identified as the candidate area for *FAP* (familial adenomatous polyposis) through an interstitial deletion in a patient with Gardner syndrome (13). Such a rearrangement should be observed through standard cytogenetic techniques, and therefore in families with the inherited trait of interest should be a kariotype of the affected patients. However, in the majority of cases the genetic defect is only the alteration of one nucleotide base, which, of course, will not be detected under g-banding staining techniques.

If cytogenetics is unable to identify the region of the genome in which the susceptibility gene is located, the next step in positional cloning is to use the technique of linkage analysis to identify the region of the genome in which the susceptibility gene is positioned. Linkage analysis works on the premise that each affected patient from each family shares a susceptibility gene. As the susceptibility gene segregates through the family, the process of independent segregation of the chromosomes and recombination along the chromosome will mean that, in addition to the susceptibility gene, the affected patients from each family will also share the genomic area surrounding the gene. Using one large, or large sets of families, linkage analysis allows the identification of this shared genomic region and hence the approximate position of the susceptibility gene. When such a region has been identified and results confirmed by subsequent studies, the position of the susceptibility gene in the genome is determined, which is named a susceptibility locus. Linkage analysis has been very successful in localising a large number of simple Mendelian traits. However, as the complexity of the genetic trait increases, the effectiveness of linkage analysis to detect susceptibility loci decreases. Familial prostate cancer is the example of how a complex genetic trait can significantly impact on the power of linkage analysis to identify susceptibility (8). Using large sample sets, a number of prospective loci have been identified, however, due to the high phenocopy rate, late age of onset, lack of a means to stratify patients into more homogenous groups and considerable genetic heterogeneity, confirmation of the linkage results has proven arduous.

By examination of the families that are linked to this susceptibility locus, recombinant events will allow the identification of an area in which all the linked families share a region of DNA. In the genetic instability in cancer, loss of heterozygosity (LOH) has greatly aided the identification of tumour suppressors and gains, through techniques such as comparative genomic hybridisation (CGH), offering the same po-

tential in identifing protooncogenes. A novel appro-ach was used by Hemminki et al (14) to map a locus on chromosome 19 in Peutz-Jeghers syndrome. This approach used CGH and LOH to identify the chro-mosome 19 region, and then used targeted linkage analysis in affected families to confirm the result. As mentioned above, the susceptibility gene was subse-quently identified as *STK11* (15, 16). A similar appro-ach was applied in the search for prospective *BRCA3* loci, and a possible locus on chromosome 13 using CGH and targeted linkage analysis (17), however, to date this linkage result has not been confirmed.

Through the examination of LOH or recombi-nant events, if this region is sufficiently small in size, if no obvious candidate gene exists, then a physical map of the area is constructed. Previously, this in-volved the tedious task of assembling a physical map of the region, using Southern blot, to identify the cor-rect area followed by construction of the physical map, by sequencing overlaying Bacterial Artificial Chromosome (BAC) clones. Once the physical map has been constructed, the genes contained in the region can be identified. Techniques such as exon trapping allow the identification of genes in the area and, once identified, these genes can be screened for mutations that segregate with the disease. However, the completion of the Human Genome Project (18), and in addition the availability of the privately funded Celera raw sequence (19), have greatly facilitated the construction of such physical maps, making the con-struction of overlapping BAC clones and sequencing redundant. The availability of the raw sequence addi-tionally offers a larger number of markers to allow fur-ther and finer restriction of an area of interest through the exploitation of the informative recombinations and LOH or CGH. Subsequently to the identification, the genes in that area can be identified, either by identification of known genes that map to that area or by prediction through computer programs, by identi-fication of Expressed Sequence Tags (ESTs) which match the sequence, gene prediction programs, and more recently, comparative sequencing between mouse and human genomes allows areas that are highly conserved to be identified (highly conserved areas are under greater evolutionary pressure and therefore are more likely to be functionally significant i.e. genes). Once the genes in that area are identified, screening the coding region in patients identifies mutations that segregate in affected families and allow the identification of the causative gene. Positio-nal cloning has been successful in identifying genes implicated in human disease, notably in the field of cancer research, *PTEN, APC, BRCA1, BRCA2, ME-NIN, PKA,* and *STK11* (20, 21) (*Figure 1*).

All of the steps in positional cloning strategy involve the utilization of molecular biology methods (such as cDNA library screening, cDNA selection, CpG island identification, exon trapping, sequence analysis, STSs, ESTs, linkage, loss of heterozygosity,
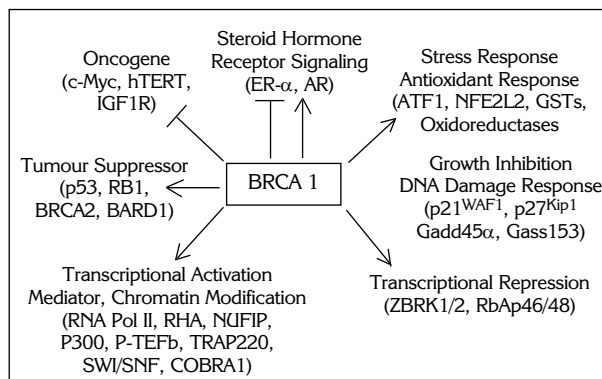


Figure 1   Summary of transcription regulatory functions of BRCA1. This figure shows the range of transcriptional pathways and individual target genes that may be regulated, in part, through BRCA1. Some of these pathways may contribute to the tumour suppressor function of BRCA1. They may also relate to normal functions of the BRCA1 protein that are not directly linked to tumourigenesis (21).

etc.) and bioinformatics methods. Our ability to find the genes involved in genetic susceptibility to many diseases, including the cancer, is increasing rapidly. The utilization of bioinformatic methods in cancer research already became a routine, owing to power-ful analytical tools and the completed human ge-nome sequence information (22, 23).

### Contribution of molecular biology in hereditary cancer diagnostics and treatment

The methods involved in molecular genetics screening in hereditary nonpolyposis colorectal can-cer (HNPCC) patients involve: DNA isolation from blood and normal colon samples, microsatellite instability testing (MSI), direct sequencing of predis-posing genes (*MLH1, MSH2, MSH6*), Western blot protein detection and immunohistochemistry (24).

DNA replication errors characterize tumours with loss-of-function mutations in mismatch repair (MMR) genes. These can be detected as microsatel-lite instability testing (MSI), which is the finding that, in the same individual, the number of repeats in a given repeating sequence of DNA varies from cell to cell instead of being constant. Several (usually five or six) such repeating sequences–called microsatellite markers – can be examined for variability (termed »instability«), indicating errors in DNA replication. MSI is termed »low« if zero or one of the markers show instability, and »high« if a high proportion of the markers is unstable. More than 90% of colorectal cancers (CRCs) in people with DNA mismatch repair gene mutations have high MSI, whereas less than 15% of sporadic CRCs do. A recent economic analy-sis compared the cost per year of life gained for three strategies for identifying cases of HNPCC: (1) geno-

typing everyone with colon cancer for MMR gene mutations (the most expensive); (2) testing every cancer for MSI and genotyping those with high MSI; or (3) applying the Bethesda criteria (family history, age, and histology) to all cases of CRC, testing for MSI on those meeting the criteria, and genotyping the subset with high MSI. Given the expense of genotyping, strategy 3 was the most cost-effective. This analysis also pointed out that the cost–benefit ratio for MMR gene mutation testing decreases dramatically if one assumes that identifying one person with MMR gene mutations leads to the offer of testing and institution of preventive measures in siblings, sons, and daughters rather than the tested individual alone (25).

### DNA microarrays as diagnostic and prognostic tools

In diseases such as cancer, many biological pathways and cell functions are irreversibly altered at the transcriptional, translational and protein level. Various technologies exist in order to investigate cancer-related modifications in the cells. One promising approach relies on microarray expression profiling. Microarrays enable a precise analysis of multiple parameters in a miniaturised format (26).

The successful clinical management of human malignancies requires an ever-evolving arsenal of both diagnostic and prognostic methods, and microarray analysis may be able to serve as a new tool that provides useful information for both. Currently, histopathologic evaluation of tumour type and grade, and pathologic and clinical assessment of a cancer's stage are the mainstays for guiding therapeutic interventions and predicting outcomes. These data are usually supplemented with information from the patient's history, the physical exam, imaging tests, and clinical laboratory assays of tumour markers (26–28).

However, even the combined use of all available clinical and laboratory information remains suboptimal for diagnosis, for predicting prognosis, and for predicting patient response to specific therapies. Tumours with identical histopathologies may progress differently, may respond differently to therapy, and may be associated with widely divergent clinical outcomes, suggesting that additional factors may be directing disease outcomes. DNA microarray technology may be a more comprehensive determinant for guiding therapeutic interventions in the future (*Figure 2*) (28).

The potential for microarray analysis to assume a significant role in cancer diagnosis and treatment selection is excellent. The importance of microarrays to future progress in oncology and other fields of medicine is supported by the recent appearance of review articles in several clinical journals (26–29). Although the technology is still evolving and its uses are still being explored, the promise demonstrated
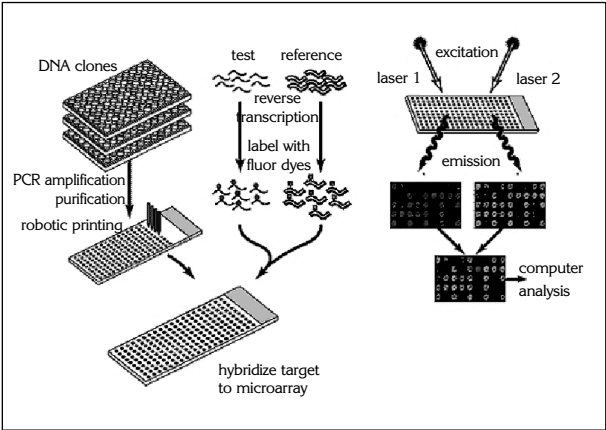
Figure 2  cDNA microarray schema: templates for genes of interest are obtained and amplified by PCR. Following purification and quality control, cDNAs are printed onto coated glass microscope slides using a computer-controlled high-speed robot. Total RNA from both the test and reference sample are fluorescently labelled using a single round of reverse transcription. The fluorescent targets are pooled and allowed to hybridize under stringent conditions to the clones on the array. Laser excitation of the incorporated targets yields an emission with a characteristic spectra, which is measured using a laser scanning instrument. Information about the clones, including gene name, clone identifier, intensity values, intensity ratios, normalization constant, and confidence intervals, is attached to each target. Data from a single hybridization experiment are viewed as a normalized ratio (that is, Cy5/Cy3) in which significant deviations from 1 (no change) are indicative of increased ($> 1$) or decreased ($< 1$) levels of gene expression relative to the reference sample. In addition, data from multiple experiments can be examined using any number of data mining tools (28).

thus far from research findings on microarrays' ability to predict prognosis of some diseases is astounding. Microarray-based studies have already identified many genes whose protein products might serve as effective biomarkers for cancer diagnoses, prognosis, and individualized treatment selection. These studies have also identified genes whose protein products may provide therapeutic targets for the progressive development of novel, more effective, and less toxic chemotherapeutic agents (29).

The gene expression profiles obtained by cDNA microarrays may help ascertain the key genetic events underlying tumour initiation, promotion, and progression. This type of genetic information may provide the foundation for the development of either universal or tumour-specific chemopreventive agents. In any event, illumination of DNA transcriptional events that are perturbed during tumourigenesis as uncovered by microarray analysis will unquestionably pave the way toward more protein-based research efforts to reveal the myriad interplay of protein func-

tions and protein-protein interactions that ultimately contribute to human tumour development (27).

## Molecular markers in clinical oncology: assays, tissues, progress and pitfalls

The promise of molecular diagnostics for cancer prevention in terms of early detection rests on two premises: assays can be developed to measure proteins, DNA, RNA or metabolites that accurately and reproducibly detect incipient neoplasias; and that this early detection will eventually result in a decrease in morbidity and mortality, and therefore benefit patients. Novel molecular technologies, including laser capture microdissection, time-of-flight mass spectrometry, DNA microarrays, tissue arrays, protein microarrays and antibody microarrays, are being developed to investigate the molecular differences between disease and normal cells and detect cancer-specific alterations in proteins, DNA and RNA in body fluids. Although literally hundreds of articles are published each year describing alterations in genes or proteins that are associated with cancer, very few result in useful molecular diagnostics for early cancer detection. Thus, there remains a critical need for new biomarkers for use in early detection and for assay methods that allow the translation of these biomarkers from the laboratory to the clinic (30, 31).

In spite of advances in diagnostics and therapeutics, cancer remains the second leading cause of death in the western countries. Successful cancer treatment depends not only on better therapies but also on improved methods to assess an individual's risk of developing cancer and to detect cancers at early stages when they can be more effectively treated. Current cancer diagnostic imaging methods are labour-intensive and expensive, especially for screening large asymptomatic populations. Effective screening strategies depend on methods that are non-invasive and detect cancers in their early stages of development. There is increasing interest in and enthusiasm about molecular markers as tools for cancer detection and prognosis. It is hoped that newly discovered cancer biomarkers and advances in high-throughput technologies would revolutionize cancer therapies by improving cancer risk assessment, early detection, diagnosis, prognosis, and monitoring therapeutic response. These biomarkers will be used either as stand-alone tests or to complement existing imaging methods (30, 31).

Molecular markers in clinical oncology can be divided into diagnostic markers, which distinguish one disease from another; prognostic markers, which are associated with the clinical behaviour of a tumour; and predictive markers, which are used to predict outcome of therapy and to aid in the selection of optimal treatment.

Diagnostic and prognostic markers, though important in clinical management, are deterministic in nature, in that the natural course of a cancer is not likely to be changed because of knowledge of that marker status. However, more excitement within the last decade has been centred on predictive markers, many of which are also the targets for specific therapeutics. What is important, of course, is that the ascertainment of these predictive markers may guide treatment selection that can change the course of a disease (31).

A troubling aspect of molecular cancer diagnostics has been previously recognized, but is still not articulated very well. Quality control, cut-off criteria, and consistent analytic formats may seem pedestrian, but are critical to achieving practical clinical impact. Unfortunately, the absence of accepted standards may lead to disturbingly high rates of false calls or, at least, uncertainty in the results. Using a variety of accepted methods for the detection of molecular perturbations, the analytical study was performed (31). Analytical results show that data discrepancies that would change clinical interpretation occurred in the range of 7% to 8%, and that the majority of the inaccuracies may be due to the clinician's not considering something as simple as the proportion of tumour cells in the sample. If significant clinical decisions are made on any one test, then this range of technical error is a source of great concern.

Although the initial reaction to these data may be one of alarm, it is actually surprising that the error rate is as low as documented given the different analytic platforms (fluorescent *in situ* hybridization [FISH], Southern blot, polymerase chain reaction [PCR]) used. Clinicians, accustomed to the reproducibility of routine laboratory tests such as serum sodium and quantitative immunoglobulin levels, are often surprised at the qualitative and relatively inexact nature of molecular diagnostics (31).

Results from molecular technologies are method-, reagent-, and operator-sensitive. For example, Southern blot analysis for *MYCN* amplification detects a band of a specific molecular weight and is sensitive to DNA degradation. Although PCR is subject to PCR amplification bias and detects only the presence of a specific fragment of the gene, it is much less sensitive to DNA degradation. A result from either PCR or Southern blot hybridization is an average of the DNA in the tumour, which includes stromal and inflammatory cells. FISH, however, like immunohistochemical analysis of tissue sections, detects single cell events in a population of cells within a tumour. Despite the fact that all techniques seek to identify gene amplification, the data outputs are sufficiently different so that the results are not always readily comparable. The most rational approach would then be to standardize one technical platform and enforce its use as the gold standard. Unfortu-

nately, sometimes even economics impede the adoption of standards. The low profit margins for some diagnostics hinder the development of standardized kits tested in rigorous (and expensive) clinical trials. Nevertheless, these issues can still be adequately addressed by organization and consensus. However, even if standardization were simple, the practical realities are much more complicated. First, molecular technologies are not stable and are highly fluid: new, more robust, more exact, and cheaper approaches emerge frequently. The evolution of the *bcr-abl* translocation assay from one based on cytogenetics to Southern blot hybridization and reverse transcriptase PCR is an excellent example of this improvement cycle. This raises the question of whether each technical improvement of a molecular test needs to be validated in a completely independent clinical trial. If this principle were applied to predictive markers in studies where the outcome may require 5 to 10 years of follow-up, few advances would be made. Second, even if tests could be standardized, biologic variability limits the convertibility of one analytic platform to another. For example, protein levels and gene amplification measure are related, but clearly different targets. Immunohistochemical analysis for *HER-2* overexpression correlates with gene amplification 70% to 80% of the time, at best. Does the 20% to 30% discrepancy nullify the utility of this test as a predictive marker? Investigators have found that, despite these discrepancies, *HER-2* overexpression by immunohistochemical analysis and *HER-2* amplification by either differential PCR or FISH were both able to distinguish the subset of node-positive patients benefiting from dose-intense chemotherapy (31).

Moreover, it is questionable what can be considered the gold standard. Biologic reality would suggest that the protein product represented by the immunohistochemical result should be more associated with tumour behaviour than gene amplification, and should therefore be considered the biochemical gold standard. For the *HER-2* marker, however, recent data suggest that FISH analysis for gene amplification is more likely to predict response to trastuzumab than the standardized immunohistochemical test. These results seem counterintuitive, but perhaps can be explained by the fact that immunohistochemistry is a less quantitative and potentially less consistent analytic test than the FISH (31).

The many ways in which a molecular marker can be defined as abnormal, when compared with normal, can also confuse the clinical interpretation of molecular results. For example, *P53* mutations with biologic consequences can be found as missense mutations anywhere in the gene that give rise to an abnormal protein, or as deletion-insertion or splice mutants that render the transcript unstable and short-lived. In addition, the same biologic outcome can be achieved by alternative abnormalities that alter downstream *p53* biology or biochemistry such as

Table III   Qualifications of a molecular test (30)

| Attribute | Questions asked |
|---|---|
| Precision in the detection of a valid target | Does it detect the appropriate molecular target? |
| Reproducibility of the test | How stable are the results? |
| Access to the necessary reagents over time | Are the reagents exhaustible? |
| Tissue composition | Is the analytical technology obsolete? |
|  | Are we testing the right tissue? |
|  | What is the fraction of tumour cells? |

murine double minute 2 (*MDM2*) amplification, or the presence of human papilloma viral oncoprotein E6 that enhances the degradation of the *p53* oncoprotein. For the *p53* status of a cancer, no single molecular test will completely define the functional abnormality and will therefore always be incomplete. This will become an important issue when therapeutics directed at abnormal *p53* pathways are developed (30, 31).

The conclusion that molecular tests are imprecise or technically unstable and should not be used is, however, inappropriate. Nevertheless, the examples discussed here should force us to develop more structured strategies in marker development and in informing the clinical community about how best to interpret these markers. Several groups have suggested standards in marker development that are reasonable and should be heeded.

The foundations of these recommendations are: precision in the detection of a valid target, reproducibility of the test, and stable access to necessary reagents over time. The work by Ambros et al (30) also highlights the importance of standardized tissue processing and the need to assess tumour and normal-tissue content (*Table III*) (30). But given the progressive importance of tumour markers in guiding therapeutic options, we should consider different ways of interpreting marker data and new approaches to speed their development and validation.

### Marker development and the new realities

The new molecular reality is that scientists are now able to generate a large number of potential diagnostic and prognostic markers with remarkable speed. The high-throughput capabilities of new technologies, such as expression and tissue arrays and
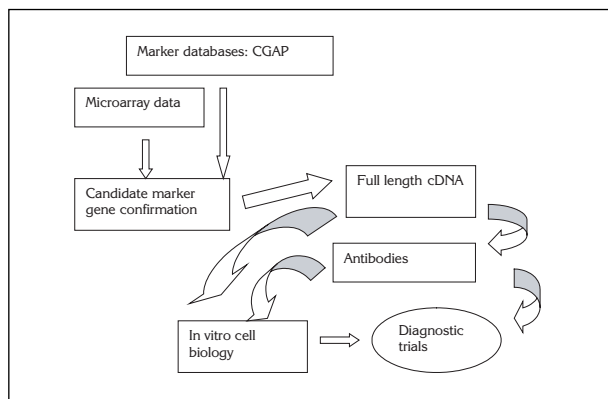
Figure 3  A model for marker development (31)

proteomic approaches, identify definitive disease markers, often without obvious mechanistic associations. In this scenario, our approach of picking one marker at a time for development is unacceptably slow. Instead, it is suggested that the following model for marker development should be considered (*Figure 3*) (31). Marker genes associated with disease or prognosis identified by high-throughput procedures or database searches (as in the Cancer Genome Anatomy Project) and then validated on a separate tissue set will need to be identified. First, the associated full-length cDNAs are cloned and recombinant proteins expressed to generate antibodies. A collection of these will be made available for any clinical trials group for testing on therapeutic trials, with an understanding that the raw data will be retained in a central data repository for later use in meta-analyses. It is estimated that for breast cancer alone there may be between 50 and 300 such markers, depending on the stringency of selection. Conceivably, all cancers can be studied in this fashion. As recently as 10 years ago, the absence of technologies such as microarrays, cDNA libraries, and antibody production would have made such a sweeping oncodiagnostic project unimaginable. Now, it is hard to imagine how cancer diagnostics can adequately be exploited without such a plan (31).

Molecular genetic testing offers important opportunities for diagnosis and assessment of genetic risk for cancer. The sensitivity of tests for rare conditions will continue to improve as additional causative mutations are identified. Genetic tests are available to determine the risk of common diseases, but these often have limited predictive value. Evaluation of the clinical usefulness of these tests will require a careful assessment of the risks and benefits of testing; the availability of specific measures to reduce risk in genetically susceptible people will be a major consideration (32).

One of the difficult challenges in the use of genetic tests is a constantly changing knowledge base. Research to evaluate interventions based on genetic risk will assume increasing importance as new tests become available. Because the development of tests to assess risk is likely to outpace the ability to reduce the risk, an ongoing dialogue involving clinicians and policymakers will be needed to develop a consensus about their appropriate clinical use (32).

# DIJAGNOSTIKA NASLEDNIH MALIGNITETA

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*Kratak sadržaj:* Naše razumevanje etiologije nastanka predispozicije za nasledna oboljenja je poslednjih godina izuzetno napredovalo. Taj napredak omogućen je pre svega naglim razvojem molekularne genetike i ispitivanja genoma, kao i njihovom primenom u humanoj genetici. Malignitet je specifičan oblik kompleksnog genetskog oboljenja. Većinu tipova malignih oboljenja karakteriše akumulacija različitih genetskih alteracija koje utiču na gene sa specifičnim patogenim potencijalom, koji su specifični za svaki tip maligniteta. Kod većine malignih tumora te genetske alteracije odvijaju se na nivou somatskih ćelija, međutim neke od njih se prenose preko germinativnog epitela i imaju ulogu u naslednoj predispoziciji za nastanak maligniteta. Budući izazov u genetici maligniteta predstavlja identifikacija gena sa visokom prevalencijom alela koji doprinose smanjenju ili povećanju rizika za nastanak maligniteta.

*Ključne reči:* dijagnostika, karcinom, nasledni malignitet

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