Journal of the Indian Institute of Science
A Multidisciplinary Reviews Journal
ISSN: 0970-4140 Coden-JIISAD

REVIEWS

One Size does not Fit All—The Future of Cancer Therapy

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Abstract | Cancer is a complex disease where normal cells of the body are transformed such that they begin to divide in an uncontrolled manner and can even invade other tissues in the body. Cancer can occur in many different tissues in the body, each requiring different forms of treatment. It is a disease that is caused by changes or mutations in genes, leading to a cascade of other genetic changes in the body. There is a high degree of genetic heterogeneity in tumors of a single type of cancer, which might explain why each patient responds to standard treatments differently. This makes it necessary to tailor treatments for cancer patients based on the molecular profiles of their tumors. This is the idea behind personalized medicine, where patients are treated based on their individual genetic changes or molecular profiles. In this paper, we look at some of the molecular profiles that are commonly used in different types of cancers, and some personalized therapies that already exist. In addition, we also attempt to predict how cancer treatment will be revolutionized by some of the new technologies that are emerging today.

Keywords: *Cancer, oncology, personalized medicine, targeted therapy, gene signatures, molecular signatures, genomics, next generation sequencing*

1 Heterogeneity of Cancer

Cancer is a disease of the cells in the body, where the normal processes of cell division and cell death go awry. Cells divide uncontrollably and live forever. It is estimated that 1 in 3 individuals develop cancer at some time in their life. According to the medical journal Lancet, it is estimated that in India alone, 555,000 people died of cancer in 2010.[1]

Cancers can develop in various tissues in the body, and can manifest themselves in different ways. Cancer is caused by mutations in genes that can further lead to other genetic and epigenetic changes. In spite of a few genetic and epigenetic changes that dominate a particular cancer, each tumor differs from the rest. Study of tumors shows that there is a lot of genetic heterogeneity among them, leading to subtype classifications on the basis of molecular profiles. We therefore need to understand cancer as an accumulation of acquired and inherited gene mutations, in addition to epigenetic changes that could be caused both by both interactions in the tissue microenvironment as well as the external environment.

Due to this heterogeneity, even within a single type of cancer, for example breast cancer, mortality and prognosis of patients differ widely. Some patients respond very well to standard surgery, chemotherapy and radiotherapy treatments, while the same treatments are ineffective in others. On the other hand, patients who do not have very aggressive forms of cancer are overtreated with standard chemotherapy regimens, which may lead to other undesired side-effects. Cancer research over the last decade has uncovered that not one, but many genes can play a role in cancer development and progression. This explains why some patients require different forms of treatment, which are not based on the phenotype or manifestation of the disease, but on their underlying genetic or epigenetic changes.

2 The Impact of Genomics Research on Cancer

The main goals of cancer therapy are focused on prevention, detection and treatment. In order to treat cancer effectively, it is necessary to look at the

Mutation: It is a permanent change in the DNA sequence of a gene resulting in a defective gene and can alter the amino acid sequence of the protein encoded by the gene resulting in the production of a non functional protein.

Prognosis: It is a prediction of the probable course and outcome of a disease or the likelihood of recovery from a disease.

Genetics: It is a branch of biology that deals with the molecular structure and function of genes, heredity, and variation in living organisms.

Epigenetics: It is the study of heritable alterations in gene expression or cellular phenotype caused by mechanisms other than changes in DNA sequence.

Gene: It is a molecular unit of heredity of a living organism and is a segment of DNA on a specific site on a chromosome that is responsible for an inheritable trait.

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entire genome as a whole, rather than individual genetic changes. In a disease like cancer, due to the heterogeneity of genetic changes, the taxonomy and classification of tumors is increasingly depending on molecular profiles, rather than morphologic characteristics. This means that genomic and proteomic profiles of patient tumors can help decide what type of treatment they should receive, and predict the prognosis of patients.

Personalized medicine is a form of therapy that uses the information about a person's genetic and protein profile to diagnose and treat a disease. This form of medicine is especially promising for a disease like cancer, which exhibits a large degree of heterogeneity. Personalized medicine aims to end the idea that one treatment can work for all patients, and attempts to find therapies that fit the individual needs of a patient.

The idea behind personalized medicine is that a patient's genomic (genetic changes in DNA), transcriptomic (RNA levels) and epigenetic (heritable changes in gene expression without changes in the underlying DNA) profiles are characterized from which target biomarkers that are known to be involved in cancer development and progression are identified. Identification of these critical genes that play a key role in carcinogenesis involves many scientific challenges. Once these gene targets are identified, drugs that target these genes can then be developed.

Several examples of personalized medicine exist in clinics today. Most of them involve some form of molecular profiling to decide the type of treatment that should be given. In some cases, a group of genes that is known to be deregulated in the specific cancer, or is a "signature" of a certain subtype of the cancer is profiled to better classify the tumor. This subtyping not only helps in deciding treatment, but in some cases is also able to predict the prognosis of the patient. In other cases of targeted therapy, a certain gene or protein is overexpressed (present at a much higher level than normal) in tumors, and a drug that targets this specific gene can be used to inhibit tumor progression. Some examples of molecular signatures and targeted therapy are discussed in the next section.

3 Molecular Signatures for Cancer Diagnosis and Prognosis

Molecular signatures of tumors aim to classify tumors into sub-classes, predict prognosis and decide treatment. They can provide a more accurate means of classification, where histopathology-based grading is ambiguous or difficult. For example, in glioma, which is a cancer of the glial cells in the brain, it is particularly challenging for pathologists to distinguish between Grades 3 and 4, which are malignant forms of glioma. Grade 3 glioma, also known as anaplastic astrocytoma (AA), is less malignant and aggressive, whereas Grade 4 glioma, also known as glioblastoma (GBM), is extremely aggressive and has very poor prognosis. We have developed a molecular signature based on microRNA expression that can accurately classify Grade 3 and 4 tumors.[2] The expression profiles of 23 microRNAs is used to accurately classify anaplastic astrocytoma (Grade 3) and glioblastoma (Grade 4) samples (see Figure 1). We have also developed a signature based on microRNA expression that can sub-divide GBM patients into high-risk and low-risk groups, and predict survival.[3] Several mRNA signatures have also been developed for GBM[4,5] that can help in prognostication and classification.

Over the last decade, several research groups have developed molecular signatures for various cancers, but very few of these have been used in the clinic. A signature that is developed in one patient cohort, very frequently needs to be validated in another patient cohort, due to the underlying heterogeneity of tumors and use of different platforms for the validation process. Unless these signatures are applicable in widely varying cohorts of patients and tested in multiple cancer centers, clinicians are unable and reluctant to use them on patients.

Despite the difficulties in translating molecular signatures from research labs to the clinic, there are a few success stories. The Onco*type DX™ Breast Cancer Assay* and the Onco*type DX™ Colon Cancer Assay* (Genomic Health, Redwood CA), MammaPrint® for breast cancer (Agendia, Amsterdam, The Netherlands) and the Breast Cancer Gene Expression ratio (HOXB13:IL17BR) test (Quest Diagnostics, Lyndhurst, NJ) are used by oncologists to help decide treatment. In glioblastoma, the methylation status of the MGMT gene is used to determine if temozolomide (a chemotherapy drug) treatment will be effective.

The Onco*type* DX® Breast cancer test is a diagnostic test that helps identify the subset of women with early-stage, estrogen-receptor positive and lymph-node-negative stage I and II breast cancer that are likely to benefit from adding chemotherapy to regimen, which includes Tamoxifen, an anti-estrogen agent. This test also helps assess the probability of cancer recurrence, which is also a factor in deciding treatment. The assay quantifies the expression of 21 genes in breast cancer tissues by Real-time PCR.[6]

Onco*type DX™ Colon Cancer Assay* is a test that provides information to clinicians on the

Genomics: It is a discipline in genetics concerned with the study of the genomes of organisms. The field includes efforts to determine the entire DNA sequence of organisms and fine-scale genetic mapping.

Proteomic: It is the study of all the proteins expressed by a given cell, tissue, or organism at a given time. Given time and under specific conditions.

MicroRNA: It is a small RNA molecule encoded by the DNA, highly conserved and can regulate the expression of genes.

Protein: It is a large molecule composed of one or more chains of amino acids in a specific order, which is determined by the RNA derived from a gene. Each protein like a hormone, enzyme, or antibody has unique functions.

Deoxyribonucleic acid (DNA): It is a nucleic acid containing the genetic instructions used in the development and functioning of all known living organisms and it consists of two long polymers of simple units called nucleotides, with backbones made of sugars and phosphate groups joined by ester bonds.

Ribonucleic acid (RNA): It is very similar to DNA, but differs in a few important structural details and is usually single-stranded, while DNA is usually double-stranded in the cell. RNA is transcribed from DNA by enzymes called RNA polymerases and it carries the information from a gene to form a protein.

Molecular signature: It is a set of genes, RNA transcripts, proteins, genetic variants or other variables that can be used as markers for a particular cell or tissue phenotype, such as a presence of a cancer.

Polymerase chain reaction (PCR): It is a molecular biology technique to amplify a single or a few copies of a piece of DNA across several orders of magnitude, producing thousands to millions of copies of a particular DNA sequence.

Real-time polymerase chain reaction (Real-time PCR): It is a molecular biology technique based on the PCR, which is used to amplify and simultaneously quantify a targeted DNA molecule.

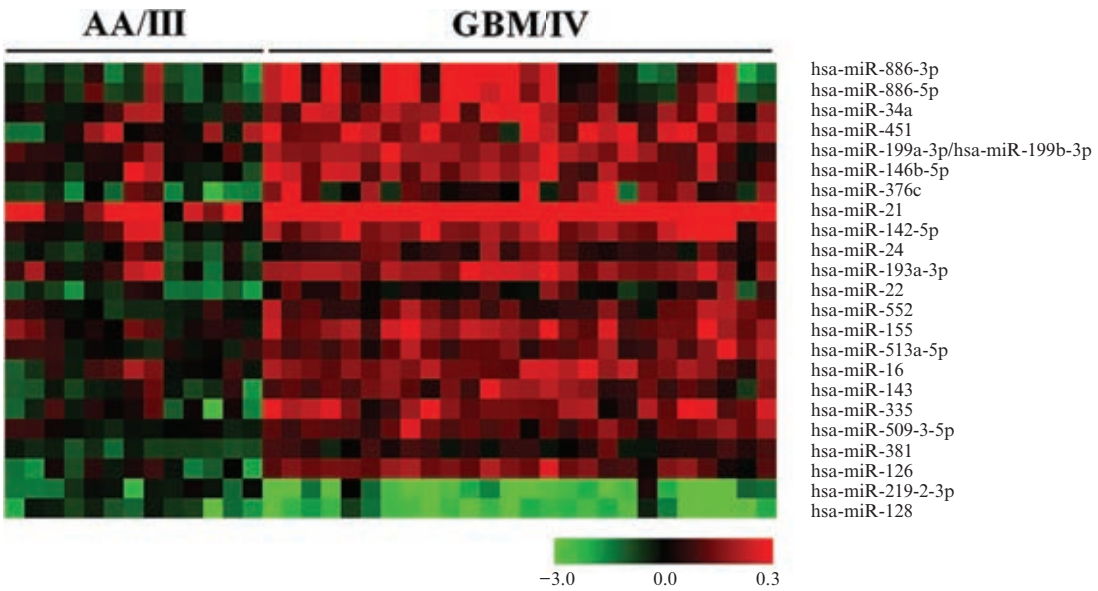


Figure 1: Heat map showing expression profiles of 23 microRNAs that can be used to classify anaplastic astrocytoma (grade 3) and glioblastoma (grade 4) samples (red indicates that the miRNA is over expressed, and green that it is under expressed).
Source: Modern Pathology (2010) 23, 1404–1417; doi:10.1038/modpathol.2010.135.

likelihood of disease recurrence in Stage II/III colon cancer and the likelihood of tumor response to standard chemotherapy regimens. It consists of a 761 gene assay based on RT-PCR.[7]

The MammaPrint® Test for breast cancer is based on microarray technology, and uses the expression of 70 genes to predict which early-stage breast cancer patients aged 61 years or younger are at high-risk for recurrence or metastasis following surgery, irrespective of estrogen receptor status or prior treatment. The outcome of the test is to predict if a patient is at a high or low risk of distant recurrence. If the patient is classified as low risk by MammaPrint®, they are treated with tamoxifen or other hormones. High-risk patients are treated with aggressive chemotherapy in addition to hormonal agents.[8]

The Breast Cancer Gene Expression Ratio (HOXB13:IL17BR) developed by Aviara DX and licensed by Quest Diagnostics is a test is used in patients that are lymph node-negative and estrogen receptor-positive to predict the risk of disease recurrence. It measures the ratio of expression of two genes, HOXB13 (overexpressed in tumors) to IL17BR (under expressed in tumors) to determine the risk factor of a patient, and helps clinicians in deciding the course of treatment.

4 Targeted Therapies for Cancer

The National Cancer Institute (NCI) of the National Institutes of Health (NIH) in the United States defines targeted therapy as "drugs or other substances that block the growth and spread of cancer by interfering with specific molecules involved in tumor growth and progression". Targeted cancer drugs that have been approved by the FDA for use in various cancers include those that inhibit molecules involved in cell growth, proliferation and angiogenesis, and those that promote death of cancer cells or stimulate the immune system to destroy cancer cells.

The aim of targeted therapy is to target cancer cells, without affecting normal cells. For example, if a protein is known to increase cell proliferation in a certain type of cancer where it is overexpressed, but is not expressed at all or at a very low level in normal cells, a targeted drug can be developed against that protein which would inhibit uncontrolled cancer cell growth, but would have no effect on normal cells.

One of the first success stories for targeted therapy for breast cancer was Herceptin (Trastuzumab), which targets the HER2 protein. HER2 is a gene that is highly expressed in some breast cancers, and is known to promote cell proliferation and migration, making the cancer highly malignant and aggressive. Trastuzumab is a monoclonal antibody that interferes with the HER2 protein, preventing cells from proliferating in an uncontrolled manner. In a routine clinical lab, patient tumors are tested for the overexpression of the HER2 through immunohistochemistry (IHC) or fluorescent *in situ* hybridization (FISH). Those patients who show an overexpression of HER2 are

Immunohistochemistry: It refers to the method of detecting antigens like proteins in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues.

Fluorescence *in situ* hybridization: It is diagnostic method utilizing fluorescently labeled DNA probes to detect a specific genetic material or nucleic acid in a cell.

Table 1: Drugs currently approved for targeted therapy for cancer.

Drug name	Chemical name	Target	Type of drug	Cancer type
Gleevec	Imatinibmesylate	ABL	Small molecule	Gastrointestinal stromal tumor, chronic myeloid leukemia, etc.
Sprycel	Dasatinib	BCR-ABL and Src family tyrosine kinases	Small molecule	CML, ALL
Tasigna	Nilotinib	BCR-ABL and other tyrosine kinases	Small molecule	CML
Herceptin	Trastuzumab	Her-2 receptor	Monoclonal antibody	Breast cancer
Tykerb	Lapatinib	HER2	Small molecule	Breast cancer
Iressa	Gefitinib	EGFR	Small molecule	NSCLC
Tarceva	Erlotinib	EGFR	Small molecule	NSCLC, pancreatic
Erbitux	Cetuximab	EGFR	Monoclonal antibody	HNSCC, colorectal
Vectibix	Panitumumab	EGFR	Monoclonal antibody	colon
Torisel	Temsirolimus	mTOR	Small molecule	renal cell carcinoma
Afinitor	Everolimus	immunophilinFk binding protein-12	Small molecule	kidney, pancreatic
Zactima	Vandetanib	EGFR, VEGF, RET	Small molecule	medullary thyroid
Zelboraf	Vemurafenib	BRAF	Small molecule	melanoma
Xalkori	Crizotinib	EML4-ALK	Small molecule	NSCLC
Zolinza	Vorinostat	HDACs	Small molecule	cutaneous T-cell lymphoma
Istodax	Romidepsin	HDACs	Small molecule	CTCL
Targretin	Bexarotene	retinoid X receptors	Retinoid	CTCL
Panretin	Alitretinoin	retinoid X receptors	Retinoid	AIDS-related Kaposi sarcoma
Vesanoid	Tretinoin	retinoid acid receptors	Retinoid	acute promyelocytic leukemia
Velcade	Bortezomib	proteosomes	Proteosome inhibitor	multiple myeloma, mantle cell lymphoma
Folotyn	Pralatrexate	RFC-1 (folate)	Antifolate	peripheral T-cell lymphoma
Avastin	Bevacizumab	VEGF	Monoclonal antibody	GBM, NSCLC, colorectal, kidney
Nexavar	Sorafenib	VEGFR, PDGFR, Raf kinases	Small molecule	renal cell carcinoma, hepatocellular carcinoma
Sutent	Sunitinib	tyrosine kinases in VEGF sigaling	Small molecule	renal cell carcinoma, GST, pancreatic
Votrient	Pazopanib	VEGFR, c-kit, PDGFR	Small molecule	renal cell carcinoma
Rituxan	Rituximab	CD20	Monoclonal antibody	B-cell non-Hodgkin lymphoma
Campath	Alemtuzumab	CD52	Monoclonal antibody	B-cell CLL
Arzerra	Ofatumumab	CD20	Monoclonal antibody	CLL
Yervoy	Ipilimumab	CTLA-4	Monoclonal antibody	melanoma

Source: Fact sheet on Targeted Cancer Therapies—NCI.

Tyrosine kinase: It is an enzyme that can transfer a phosphate group from Adenosine 5-triphospahte (ATP) to a protein by a process called phosphorylation, which forms a important mechanism of communication of signals within the cell.

Chromosome: It is an organized structure of DNA and protein found in cells and is a single piece of coiled DNA containing many genes, regulatory elements and other nucleotide sequences.

treated with Herceptin in combination with other chemotherapy or hormonal agents.

Gleevec is another cancer targeted therapy drug that has been around since the 1990s. It was originally developed as a drug against the tyrosine kinase ABL that is activated in chronic myeloid leukemia (CML). The Philadelphia chromosome or Philadelphia translocation, which is commonly seen in CML, is a result of the fusion gene BCR-ABL that is formed when a portion of chromosome 9 and a portion of chromosome 22 break off and switch places. ABL is a tyrosine kinase that is involved in signalling pathways controlling cell proliferation. In the BCR-ABL fused gene, ABL signalling is constitutively active, promoting proliferation. Gleevec works by targeting the ABL protein and inactivating the signals that increase cell proliferation.

In the last decade, there have been several targeted therapies that have been approved for various cancers. Table 1 has a list of these therapies and the cancers they have been approved for. Patient tumor samples are tested for specific targets, to decide if the patient would be a good candidate for the targeted drug in combination with other therapies.

5 Applications of Next Generation Technologies to Cancer Personalized Therapy

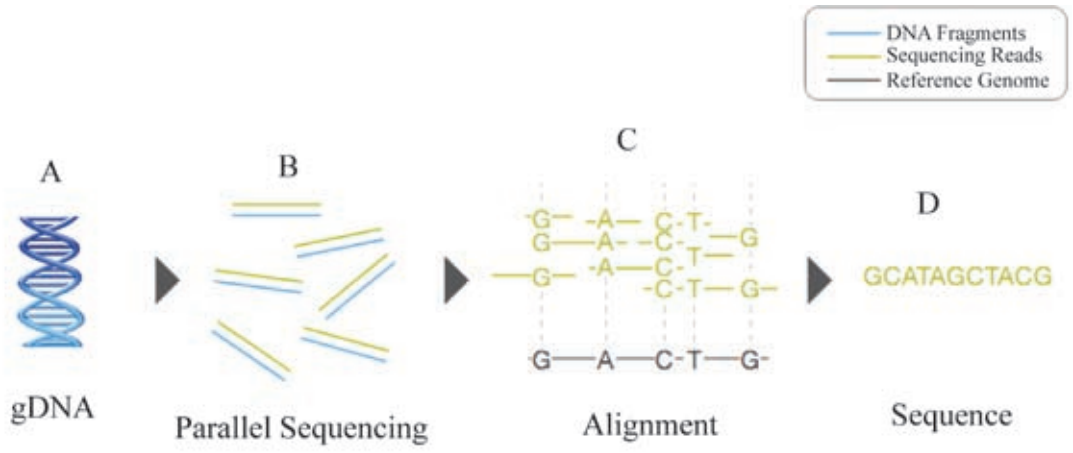
When the human genome was sequenced in the early 2000s, the technology used was Sanger sequencing, which was costly and time-consuming. Sanger sequencing is frequently referred to as "first generation sequencing". "Second generation" or Next generation sequencing (NGS) technologies overcome the scalability limitations of Sanger sequencing by allowing millions of sequencing reactions to happen in parallel. Next generation sequencing methods generate millions of short reads, which then need to be aligned to generate the entire sequence (see Figure 2). There is a lot of flexibility to fine-tune the degree of resolution for an experiment. For example, in order to detect variants with a high confidence, a scientist would design an experiment to have the genome sequenced at 50x coverage, which means, that every base, on average, was covered by 50 short reads. On the other hand, if the experiment involves sequencing a large number of genomes, as in the 1000 genomes project to identify common SNPs, the scientist could opt for a low coverage of 5x for each genome and look for common variants across multiple samples.

Next generation sequencing (NGS) technologies promise to change the landscape of personalized medicine and take it to a new level. The cost of sequencing the human genome in the early 2000's was around 100 million dollars, whereas the cost of sequencing a single individual's genome has now come down to a few thousand dollars (See Figure 3). With the cost of genome sequencing coming down rapidly, it is possible to envision a day not too far away when every individual can have their genome sequenced as part of routine clinical lab tests, and the results are used by doctors to tailor treatments for them. This is especially true for a disease like cancer that exhibits a high degree of heterogeneity. Doctors can decide the combination of drugs that are likely to work for the specific patient based on his genomic, epigenomic and transcriptome profile.

Transcriptome: It refers to a set of all RNAs expressed in a given organism or tissue or cell at a given a time.

6 Future Path for Personalized Medicine in Cancer

With the advent of new technologies that are bringing down the cost of sequencing human genomes, it is no longer a fantasy to imagine that every individual can have their genome sequenced. In the future, it is possible to imagine that everyone carries a smart card with their sequenced genome on it, and doctors can then use this information to



A. Extracted gDNA
B. gDNA is fragmented into a library of small segments that are each sequenced in parallel
C. Individual sequence reads are reassembled by aligning to a reference genome.
D. The whole-genome sequence is derived from the consensus of aligned reads.

Figure 2: An overview of next generation sequencing technologies.
Source: www.illumina.com

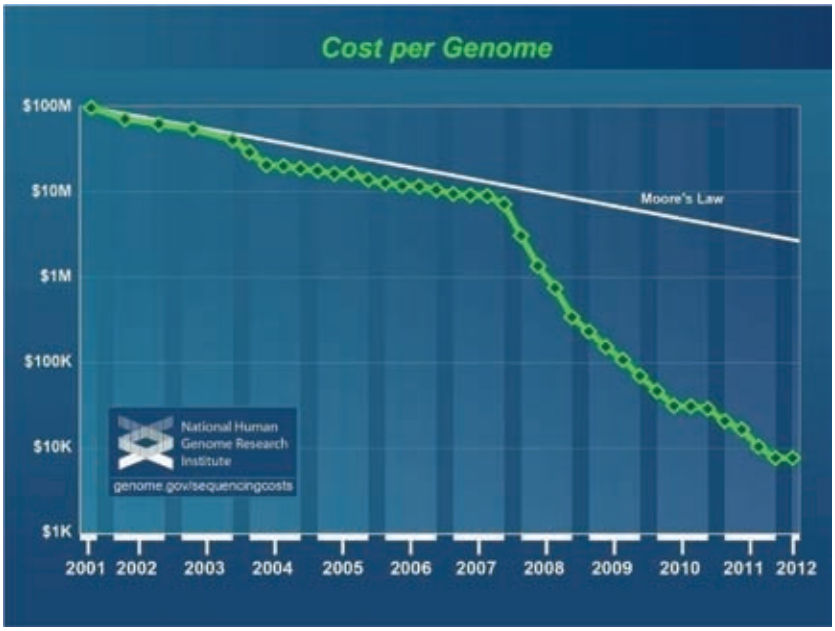


Figure 3: Cost of sequencing human genomes over the past decade. In 2001, the cost of sequencing a whole human genome was around $100 million. The cost has steadily decreased and the current cost of sequencing a human genome is now less than $10,000.
Source: NHGRI, NIH.

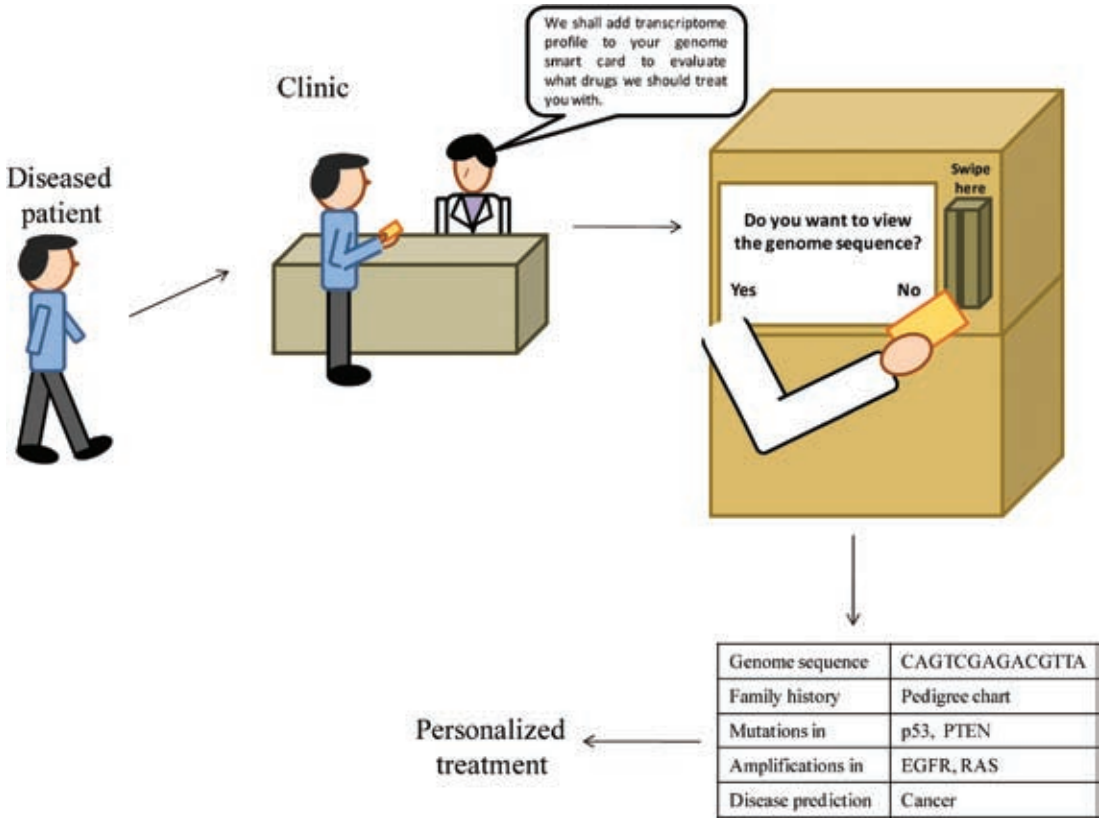


Figure 4: A future that uses genome smart card which helps in personalized treatment.

determine possible risk factors and tailor therapies that would be effective for them. In the context of oncology, it is possible that in addition to using information from the genome, the doctor would also order a transcriptome profile of the tumor sample. This would help the doctor decide which targeted therapies, if any would be effective to combat the disease, and what combination of drugs to use (Figure 4).

While this reality is possibly not far away, we should also be cautious to recognize the challenges we would need to face on the way. While many gene signatures and biomarkers have been identified in cancer research labs around the world, most of them have not been translated into practice in the clinic. The reasons for this are manifold—lack of validation in multiple patient cohorts, variability in results, lack of standardization in clinical lab testing and the underlying nature of the disease that makes it difficult to find common features across large patient cohorts. In addition, translation of research into the clinic requires a concerted effort and collaboration between bench scientists, computer scientists, bioinformaticians, biostatisticians and clinicians, so that meaningful results are obtained. The initial costs of setting up the infrastructure required to support personalized medicine are also a consideration, especially in developing countries like India. There are also ethical issues to be considered, such as, who would have access to information about an individual's predisposition to a certain disease or condition, especially with respect to insurance companies and employers.

It is clear that personalized medicine, if it becomes a reality, has a lot of benefits for patients, with better treatment and fewer side-effects. It is also a boon for care-givers, who can customize treatments for their patients, based on their individual conditions. In addition to treatment, doctors can also advise their patients on lifestyle changes and preventive measures that they can take, if they are predisposed to certain diseases. Despite the challenges, the rewards are enormous, and if the promise of personalized medicine is fulfilled, we can all look forward to better and healthier lives.

Acknowledgements

Authors thank DBT, Government of India for financial support. Infrastructural support by funding from ICMR (Center for Advanced studies in Molecular Medicine), DBT, DST (FIST) and UGC to department of MCB, Indian Institute of Science is acknowledged. We also thank Irene Rosita Pia Patric for the technical assistance. K.S. is a J. C. Bose Fellow of the Department of Science and Technology.

Received 7 September 2012.

References

1. Dikshit, R., et al., *Cancer mortality in India: A nationally representative survey*. Lancet. **379**(9828): p. 1807–16.
2. Rao, S.A., V. Santosh, and K. Somasundaram, *Genome-wide expression profiling identifies deregulated miRNAs in malignant astrocytoma*. Mod. Pathol. **23**(10): p. 1404–17.
3. Srinivasan, S., I.R. Patric, and K. Somasundaram, *A ten-microRNA expression signature predicts survival in glioblastoma.* PLoS One. **6**(3): p. e17438.
4. de Tayrac, M. et al., *A 4-gene signature associated with clinical outcome in high-grade gliomas.* Clin. Cancer Res. 2011. **17**(2): p. 317–27.
5. Colman, H. et al., *A multigene predictor of outcome in glioblastoma.* Neuro. Oncol, 2010. **12**(1): p. 49–57.
6. Cobleigh, M.A. et al., *Tumor gene expression and prognosis in breast cancer patients with 10 or more positive lymph nodes*. Clin Cancer Res, 2005. **11**(24 Pt 1): p. 8623–31.
7. Clark-Langone, K.M. et al., *Biomarker discovery for colon cancer using a 761 gene RT-PCR assay*. BMC Genomics, 2007. **8**: p. 279.
8. van 't Veer, L.J. et al., *Gene expression profiling predicts clinical outcome of breast cancer*. Nature, 2002. **415**(6871): p. 530–6.

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