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The Pharmaceutical Applications of Next Generation Sequencing in Oncology Drug Designing and Development

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Abstract

Intensive efforts and international collaborations have been performed and are still ongoing in the fight to beat cancer, and many successes have been achieved as a result of hardworking scientists. Although cancer still exists, new information is learned every day, and research continues to be published containing new theories and findings. Cancer research is characterized by the use of advanced technologies that play a role in accelerating results, which then translates into clinical practice to improve the concept of personalized medicine. Currently, next-generation sequencing [NGS] is one of the most advanced technologies used in cancer research due to its ability to provide valuable information from genomic sequencing. Not only is it capable of examining genes but it can also detect and target expected mutations in certain clinical situations and find unexpected sequence variations without consuming substantial time or effort; therefore, NGS provides clinicians with a better understanding of cancer mechanisms. This article demonstrates the pharmaceutical applications of NGS, from tumor markers to pharmacogenomics and targeted therapy, precision medicine, vaccinology, biopharmaceutics, polypharmacology, toxgnostics, and pharmacoepidemiology.

Keywords: Next generation sequencing; NGS; Cancer; Pharmacogenomics; Precision medicine; poly pharmacology; Toxgnostics; Pharmacoepidemiology; Personalized biomarker

Introduction

Genes, which are present in every cell of the body, are composed of deoxyribonucleic acids, or DNA. DNA is composed of the following four nucleotide bases: adenine, guanine, cytosine and thymine [AGCT sequence]. Contrary to popular belief, the DNA sequences of all individuals are 99.9% identical and only 0.1% unique.

DNA controls vital cell processes in different ways, such as the control of cell growth, division and death, by making specific proteins; therefore, it is important for each gene to have a specific coding "sequence", allowing all of the subsequent proteins to be made properly. Genetic variations commonly occur, but not all genetic variations cause disease.

Genetic variations are simply differences in DNA sequences, and they can be observed at every genetic level in DNA—in the genes, chromosomes, and proteins and in their functions. Therefore, when discussing the variations in a DNA sequence, one is generally referring to mutations that are present at a level of less than 1% in a population.

Generally, two types of genetic mutation events create all forms of variation:

1- Single base mutations [single nucleotide polymorphisms, or SNPs] or

2- The insertion or deletion of one or more nucleotides.

Therefore, if an individual has a mutation (including SNPs, structural variations, copy number variations [CNVs], somatic copy number aberrations [CNAs], insertions, deletions, differentially expressed genes, differentially expressed isoforms, translocations, expressed variations, predicted gene fusions), the presence of these mutations will affect transcription and can alter the protein structure and function, leading to complex diseases, such as cancer.

The beginning of all types of cancer, which is a multifactorial disease that is characterized by its intrinsic heterogeneity [1], begins with mutant cells that create abnormal proteins with altered functions or no protein at all, which then begins the carcinogenic process—initiation, promotion, progression and metastasis. In other words, at the normal cellular scale, there is a balance between growth promoters and inhibitors, but in cancer this balance is lost, leading to sequential processes and the phenotypic hallmarks of malignancy. As proliferation increases, difficulties in differentiation lead to the immortalization of the cells and resistance to normal apoptosis.

The human body normally has the ability to correct the majority of these mutations, but in cancer, this ability differs because the mutations begin to accumulate in vital genes, resulting in alternations to major cellular pathways. As more accumulations occur, the cancer worsens.

Some types of cancer can arise from normal tissues or from specialized cells types, but other types do not fit into the major classifications, such as melanomas, which arise from the neural crest, a primitive embryonic structure [2]. It is worth noting that the information provided by a mutant gene will be different from the normal gene. This clearly shows that there is an increased need to use advanced techniques to read DNA sequences and to detect mutations, to better understand these mutations and to determine the best personalized cure based on the results.

Unfortunately, due to the complexity of cancer, there are currently no drugs that can eliminate cancer cells in patients. Many anticancer therapies are taken in combination and at different cycles, and the majority are toxic, with a narrow therapeutic index and the ability to cause morbidity. The first generation of anticancer therapies began in the 1950s; these therapies were mostly cytotoxic [3] and caused several side effects in cancer patients.

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Another challenge that lies in cancer treatment is the ability to detect and diagnose cancer during the early stages. The early detection of cancer leads to a significant increase in the cure rate. However, the majority of cancers are detected during their late stages, when treatment opportunities are limited, resulting in poor survival [4]. Therefore, there is a need to utilize more novel approaches for cancer management that allow the targeting of cancer cells without causing harm to normal cells.

The general approaches for cancer management and therapy lie in destroying or removing cancer cells, either via surgery, irradiation, or chemotherapy. The combination of these approaches shows many benefits for improving a cancer patient's quality of life and increasing their survival rate.

Each approach has is important in its own way. Surgery plays multiple roles in cancer management, including prophylaxis if the organ is not crucial for survival; diagnosis, by taking a biopsy from a cancer patient; staging and measuring the extent of the tumor, including allowing the determination of the type of tumor, the growth extent, the size, the nodal involvement and the tumor distance and regional spread; as a definitive or curative treatment, by removing the entire tumor and the lymph nodes that are associated with it; as palliative care to reduce the symptoms and distress; as an adjuvant or supportive therapy, such as a tracheostomy and feeding tubes; as a reconstructive or rehabilitative therapy to reduce deformities; and as a salvage treatment for local disease recurrence, such as via a salvage radical cystectomy or mastectomy [5].

Radiation also plays many roles because both cancer cells and normal cells are affected in cancer. The primary function of radiation is as a curative treatment. For example, some cancer types, such as skin cancer, prostate cancer, early stage larynx cancer and Hodgkin's lymphoma, can only be cured with the use of radiation. Additionally, radiation serves as a form of adjuvant treatment after definitive surgery or as a palliative treatment. This approach has undergone substantial developments, such as the use of intensity-modulated radiation therapy [IMRT], image guided radiotherapy [IGRT], radioimmunotherapy, stereotactic radiosurgery [SRS], and stereotactic radiotherapy [SRT] [5].

Chemotherapies have proven to be effective during the early stages of cancer because they interfere with DNA synthesis steps and tumor replication. They play roles as a form of adjuvant treatment to reduce micrometastases and cancer recurrence or as a neoadjuvant to shrink a tumor [5]. An example is the multi-drug treatment protocol used in Hodgkin's lymphoma. ABVD, which consists of doxorubicin, bleomycin, vinblastine, and dacarbazine, works via intercalation, breaking the DNA strand, and microtubule inhibition [2].

Next-Generation Sequencing [NGS]

One of the more recent advances in genomic technologies is the development of high-throughput sequencing platforms. These technologies have become powerful tools in cancer research.

High-throughput sequencing is based on 3 main steps: using a solid support to immobilize the DNA samples, using automated fluidic devices for cyclic sequencing reactions and using imaging to detect molecular events [6].

First-generation sequencing [Sanger sequencing] is considered the gold standard for sequencing, has been used as a powerful tool for genomic analysis in the past, and is still in use for the validation of high-throughput sequencing analyses. High-throughput sequencing encompasses all of the terms used to describe a number of sequencing technologies, including Illumina [Solexa] sequencing, Roche 454 sequencing, and SOLiD and Ion Torrent.

NGS is flexible because it can be combined with other technologies and supplementary tests. TAm-seq, which is a method developed by Forshew et al., has the ability to detect TP53 mutations by combining primer design and NGS [7]. NGS has many advantages over Sanger sequencing. For example, during sample preparation, less DNA is required for the preparation of the sequencing libraries; normally 3 μg of DNA is required to sequence the BRCA1 and BRCA2 genes using Sanger sequencing, but only 500 ng of DNA is required for chip-captured NGS sequencing [8].

NGS provides the key to understanding the process of oncogenesis in a genetic manner, due to its ability to provide information regarding a genomic sequence. NGS is capable of both detecting expected mutations in a certain clinical situation and determining unexpected sequence variations, therefore playing a significant role in treatment and in other therapeutic purposes [9].

Applications of NGS

Tumor markers

The annual report from the American Cancer Society provides the number of new cancer cases and deaths each year. In 2014, approximately 1,665,540 new cancer cases were diagnosed, and there were 585,720 cancer deaths. Anyone can develop cancer, but a substantial proportion of cancers can be prevented [10] if diagnosed accurately and early using proper methods and not at the advanced stages of cancer propagation.

What makes cancer so difficult to detect is that the lifespan of most cancers is characterized by silence, which means that all of the changes that occur in normal cells during their conversion into cancer cells, such as dysplasia, hyperplasia and pleomorphism, occur silently [5].

Additionally, a cancer diagnosis can be difficult because the patient may be asymptomatic or have signs and symptoms of other non-cancerous diseases; in this case, a diagnostic test is required. Therefore, a biopsy is performed, which requires the surgical removal of a piece of the suspected cancerous region [5].

Currently, a new interest of scientists and researchers is the productions of genome-based blood tests. One of the major benefits of these advanced tests is the detection of tumor markers, which are the materials secreted by a tumor. The development of personalized blood tests for cancer using whole-genome sequencing has been a focus at Johns Hopkins University. Additionally, NeoGenomics, a leading provider of cancer-focused genetic and molecular testing services, announced on Friday, July 11, 2014 that it had launched 23 new types of cancer profiles based on NGS (Figure 1).

The history of the development of tumor markers began during the 1960s when enzymes, hormones and serum proteins were utilized as tumor markers for therapeutic purposes [11].

During the late 1970s, the first tumor marker was discovered, which contained carcinoembryonic antigen [CEA] or carcinoembryonic protein [12], providing new hope and ambition in the fight against cancer by gaining more valuable information regarding the properties of tumor markers and their mechanisms of action. In addition, this discovery provided insights on how to translate this information into clinical practice, especially in cancer patient management.

Tumor markers can be discovered by detecting SNPs, which are the most commonly occurring mutations in the population. Using the

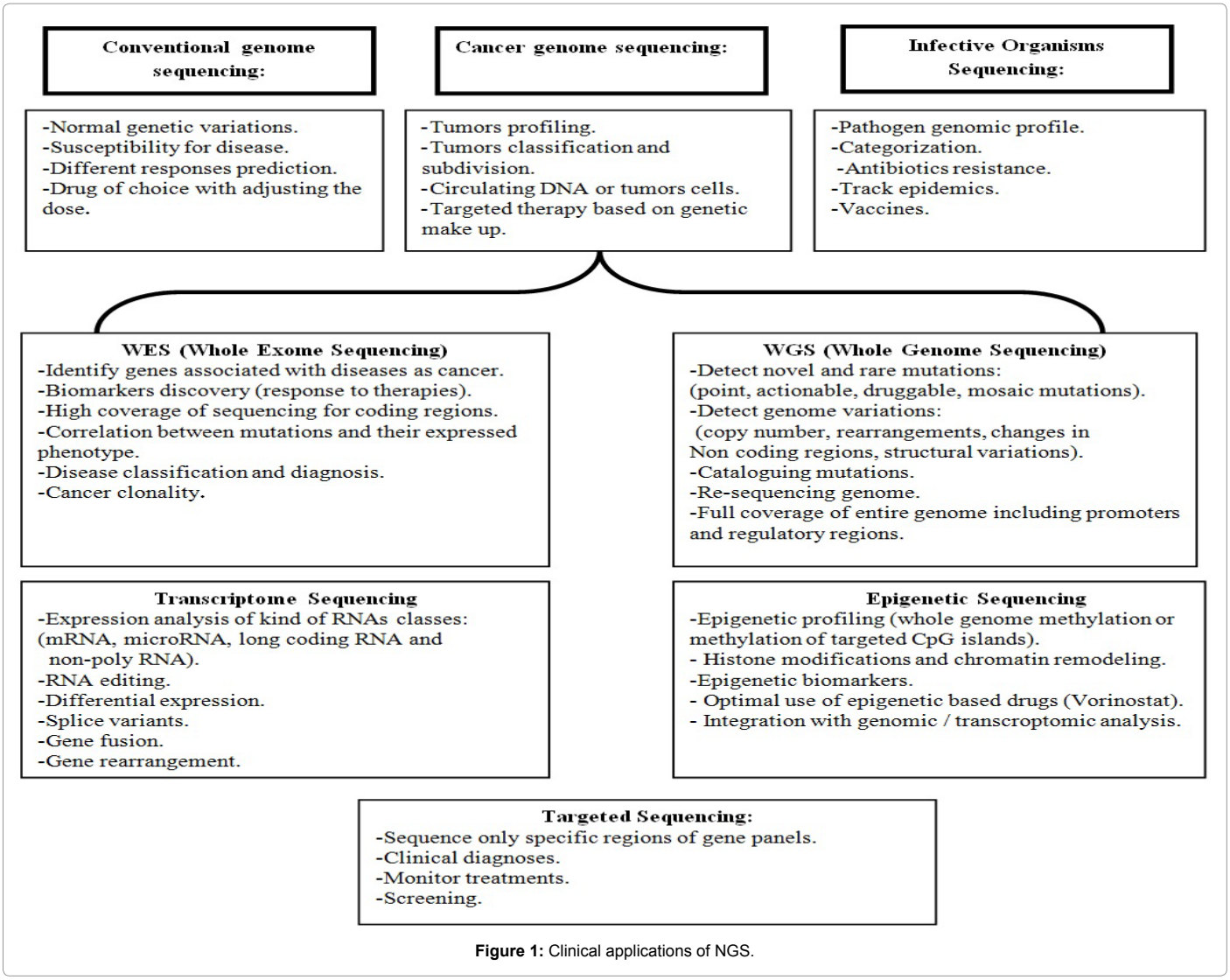

Figure 1: Clinical applications of NGS.

following sequence as an example: ATG CAA to ACG CAA, if a single substitution occurs from T to C, the entire nucleotide sequence will carry the disease because the change in the sequence, or the mutation that occurred, will produce an abnormal protein. Therefore, this SNP is the marker for this disease.

The next step is the development of strategies for the discovery and utilization of personalized tumor markers by using individual genetic profiles. Thus far, personalized tumor markers have achieved many successes in different fields of oncology, including early cancer detection, screening, diagnosis, prognosis, targeted therapy, therapeutic response, and monitoring and recurrence (Table 1).

Pharmacogenomics [PG]

Pharmacogenomics is the combination of pharmacology and genomics. This field focuses on the study of how drugs respond differently from person to person due to genomics [13] and how genotype-phenotype information can be utilized in individualized medicine.

The major benefits of PG are as follows:

1. The development of drugs to maximize their therapeutic effects.

2. The ability to design more accurate dosage methods based on an individual's genetic profile rather than using traditional measurements, such as body weight and age [13].

3. The ability to identify the responders /non-responders for different drugs and to determine the risk factors for that drug.

To apply these benefits in real life, one must know the main principles and methodology used in PG, which can be summarized as follows:

1. The use of classical genetics techniques [heritability] by applying Mendelian genetics.

2. The use of genome-wide association studies [GWAS] to examine genetic differences in different individuals, which is performed by comparing the DNA sequences between two groups—the first group is affected by the disease, and the second group is the control without the disease. If the results indicate the presence of a SNP more frequently in one group than in the other, it is likely associated with the disease.

Cancer type	Tumor marker	Clinical findings
Bladder cancer	ICAM-1/VCAM-1	Higher in all cancer patients than in controls.
	sFas ligand	Higher levels of sFasL predict early recurrence in Ta bladder carcinoma.
Brain cancer	bFGF	Indicate presence of tumor.
	VEGF	Indicate presence of tumor.
Cervical cancer	IL-2R	Higher levels in 50% of patients with squamous cell carcinoma.
	SCC	Higher levels in 67.5% of patients with squamous cell carcinoma.
Colon cancer	Angiogenin	High levels correlated with cancer progression.
	E-selectin	Elevated in colon and breast cancer patients.
Endometrial cancer	P53	Adjunctive test for gynecological cancers preoperative.
	IL-2R	Higher levels in 51.4% of patients with endometrial.
Esophagus cancer	IL-2R	Significantly increased in esophagus cancer patients.
	P53 AB	Detected in 43% of esophagus cancer patients.
Stomach cancer	c-erbB-2	Correlated with HER-2/neu tissue over expression.
	COX-2	Over expression is associated with high levels of prostaglandin E2 biosynthesis and ogenesis.
Breast cancer	TGF-β	Highly expressed in primary breast tumors.
	ICAM-1	High levels in 96% of sICAM-1 in breast cancer patients.
Leukemia	TNF-α	Therapy clinical efficacy, indicator of residual disease presence.
	IL-2R	Only elevate it in relapse patients, interferon therapy response prediction.
Lung cancer	SVCAM-1 and other CAMs	Detect abnormal levels in SCLC.
	TNF-α,TGF-β	Show favorable prognosis for positive lung cancers.
Pancreatic cancer	CgA	High levels in 99% carcinoid tumors .elevated in endocrine pancreatic tumor, multiple endocrine neoplasia 1 syndrome.
	P53 mutant protein	Detected in 50% of the patients.

Table 1: The critical roles of tumor markers in different cancer types[11].

3. The use of association studies, which consume significant time and effort, especially during follow-up.

These studies connect phenotypes with genetic markers and are based on three primary strategies:

1. Case-control association studies, 2. family-based association studies, and 3. population-based association studies.

Different drugs have different effects in different populations. This indicates that different patients may have the same symptoms, the same findings, and the same disease, and although they are taking the same drug at the same dose, they show different effects and reactions. Therefore, different people react differently to drugs, which are why they are classified as responders, non-responders, or toxic responders due to these individual variations. A percentage of patients benefit from approved drugs, but a portion of drugs have failed in clinical trials, and more importantly, many approved drugs have been removed from the market due to their serious adverse effects.

Some possible reasons for these individual variations may be ethnicity, age, environment, gender and genetic variation. In the field of drug application, the genetic variations that are present in critical genes that are responsible for metabolizing certain drugs and transporting or targeting drugs will influence a drug's activity (Table 2).

For example, in 5-fluorouracil (5-FU), which uses dihydropyrimidine dehydrogenase [DPYD] as a rate-limiting metabolizing enzyme, the presence of a mutation at DPYD*2A will cause partial DPD deficiency, leading to 5-FU toxicity [14-31]. Therefore, other alternatives must be considered, such as changing the drug or changing the dose, depending on the patient's condition. The FDA provides this information on the drug label in the warning section. The Theraguide 5-FU blood test has begun to be used to allow customized management and to minimize the patient's risk for an adverse reaction to 5-FU-related chemotherapies.

In addition, both germline mutations and somatic mutations play roles in drug response. Germline mutations have been used for predicting drug toxicity and efficiency [32] and to develop new or existing therapeutics [33]. Somatic mutations have been used to optimize certain anticancers [32] and to provide accurate drug targets [33]. For example, non-small cell lung cancer sequencing has shown that EGFR tyrosine kinase inhibits sensitivity resistance, which has resulted in the use of routine EGFR sequencing for therapeutic purposes [34].

The application of PG in the drug field begins by targeted identification and discovery, followed by the optimization of a compound, clinical trials, and finally, the launch of a product. Currently, with the huge development in genomic personalized medicine, NGS plays a critical role in designing new therapeutic strategies based on the genetic profile of each individual.

Because cancer is genetically unique, using personalized therapy in cancer is more applicable and should be required. To better understand the concept of designing new therapeutic strategies, DNA sequencing using NGS can detect mutations that will lead to the design of drugs that specifically target these mutations; these drugs are based on gene-targeted nucleic acids.

The blocking of apoptosis, cell proliferation, tumor angiogenesis, tumor invasion, tumor suppressor gene dysfunction, increased tumor cell lifespan, DNA synthesis arrest, and drug resistance can all lead to cancer. All of these are carcinogenic effects, the main cause of which is the presence of mutations; therefore, detecting mutant genes will impact the outcome of the therapy (Figure 2).

An example of a target gene involved in proliferation is the Ras oncogene family [K-ras, H-ras, N-ras]. The Rasfamily are proteins involved in transmitting signals within cells from tyrosine kinase receptors to the cell nucleus, and they regulate a wide range of processes [35]. The presence of a mutation will cause the loss of the cell's dephosphorylation ability, leading to an increased proliferation rate and cancer cell survival (Figure 3) [36,37].

Selected Germline Genetic Variants			
Drug	5-FU	6-MP	Irinotecan
Gene	DPD	TPMT	UGT1A1
Role	Rate limiting metabolicenzyme	Major inactivation enzyme	Major enzyme for SN-38Glucuronidation.
Mutations	DPYD*2A= partial DPD deficiency lead to 5-FU toxicity[14].	TPMT*2, TPMT*3A, /TPMT*3C = Low TMPT activity [3] which may associated with high myelotoxicity risk [15].	UGT1A1*28=SN-38accumulation leading to Irinotecan toxicity [3].
FDA Label	yes	yes	yes
LabelingSection	Warnings	Dosage and Administration,Contraindications, Precautions, Adverse Reactions, Clinical Pharmacology	Dosage and Administration, Warnings, Clinical Pharmacology
Test	Theraguide 5FU	TPMT Testing	InvaderR UGT1A1
Url	http://www.myriad.com/products/theraguide-5-fu/	http://labtestsonline.org/understanding/analytes/tpmt/tab/test/	http://www.invaderchemistry.com/invader_applications/invader-ugt1a1.html
Selected Somatic Genetic Variants			
Drug	Crizotinib	Vemurafenib	Gefitinib/Erlotinib
Gene	EML4-ALK	BRAF V600E	EGFR-tumor activating
Role	receptor tyrosine kinase that is aberrant in a variety of malignancies	serine-threonine protein kinases which involved in many cellular processes. Mutant BRAF has been implicated in the pathogenesis of several cancers.	receptor tyrosine kinases, induces a conformational change that facilitates receptor homo- or heterodimer formation.Activated EGFR then phosphorylates its substrates, resulting in activation of multiple downstream pathways within the cell.
Mutations	-Resistance in C1156Y, L1196M mutations present [16-17]. -Response in EML4-ALK rearrangement present [18-21].	- Response in BRAF V600E mutation present [22].-Resistance in MAPK pathway reactivation [23,24].	-Response in EGFR-activating mutation present [25-29]. -Resistance in T790M mutation present [30,31].
FDA Labeling Section	Indications and Usage, Dosage and Administration, Drug Interactions, Warnings and Precautions, Adverse Reactions, Clinical Pharmacology, Clinical Studies	Indications and Usage, Warning and Precautions, Clinical Pharmacology, Clinical Studies, Patient Counseling Information	Indications and Usage, Dosage and Administration, Clinical Pharmacology, Clinical Studies
Test	theVysis ALK Break ApartFISH Probe Kit	cobas[®] 4800 BRAF V600 Mutation Test	cobas® EGFR Mutation Test
URL	http://www.abbottmolecular.com/us/products/oncology/fish/lung-cancer/vysis-lsi-alk-dual-color-break-apart-rearrangement-probe.html	http://molecular.roche.com/assays/Pages/cobas4800BRAFV600MutationTest.aspx	http://molecular.roche.com/assays/Pages/cobasEGFRMutationTest.aspx

Table 2: The effect of genetic variations on anticancer drugs and how the FDA uses this information in drug labeling and tests that are approved to detect these mutations.

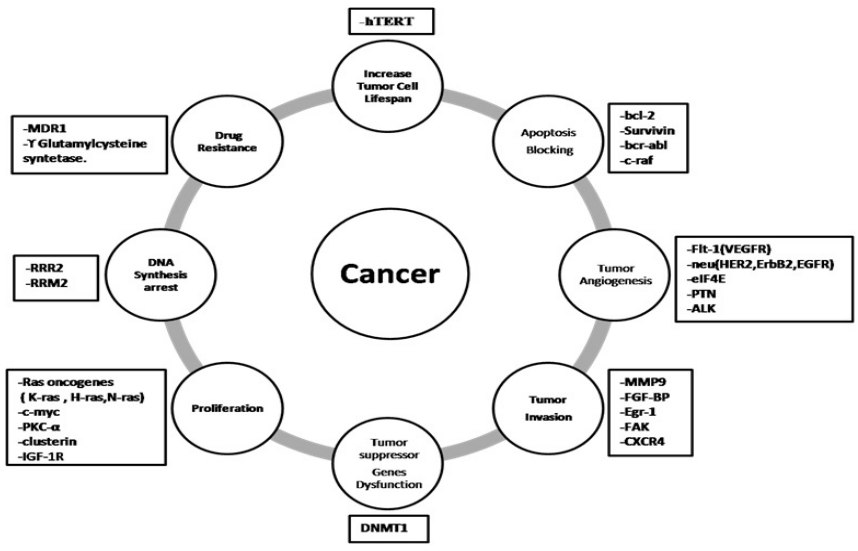

Figure 2: Carcinogenesis Events and Target Genes

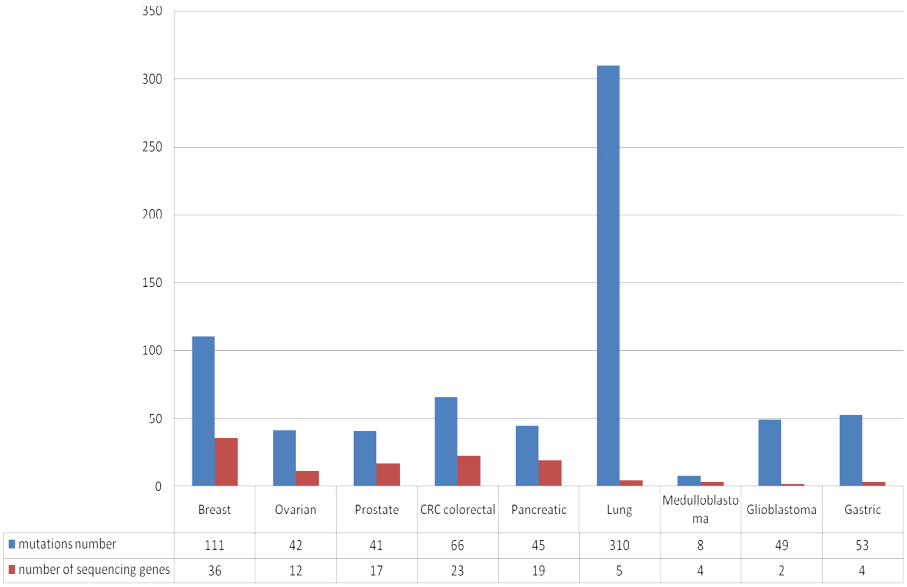

Figure 3: A depiction of the need to apply NGS in cancer showing the number of mutations in different types of cancers and the number of sequenced genes [37].

Cancer Type	Mutation	Type /Location fMutation	Implications for Targeted Therapeutics				
Chronic myeloid leukemia (CML)	BCR-ABL1 Fusions	t[9;22][q34.1;q11.21] and other translocations resulting in the BCR- ABL1 fusion gene	Imatinib	Dasatinib	Nilotinib	Bosutinib	Ponatinib
			Confers sensitivity	Confers sensitivity	Confers Sensitivity	Confers sensitivity	Confers sensitivity
	BCR-ABL1 Y253H	P-loop region of the kinase domain [Exon 4]	Confers reduced sensitivity	Retains sensitivity	Confers reduced sensitivity	Unknown at this time	Unknown at this time
	BCR-ABL1 V299L	SH3 contact region of the BCR-ABL1 kinase domain [Exon 5]	Confers reduced sensitivity	Confers reduced sensitivity	Retains sensitivity	Unknown at this time	Unknown at this time
	BCR- ABL1 T315I	Kinase domain [Exon 6]	Confers reduced sensitivity	Confers reduced sensitivity	Confers reduced sensitivity	Confers reduced sensitivity	Retains sensitivity
Basal Cell Carcinoma (BCC)	SMO D473H	At the boundary between the 6th transmembrance domain and an extracellular domain	Vismodegib				
			Hypothesized to confer decreased sensitivity				
Breast Cancer	AR Expression		Androgen Receptor Modulators and Antagonists		Estrogen Receptor Agonists/Antagonists		Aromatase Inhibitors
			Confers increased sensitivity		Unknown at this time		Unknown at this time
	ER Expression		Confers increased sensitivity		Confers increased sensitivity		Unknown at this time
	HER2 [ERBB2] Amplification		Trastuzumab	Ado- Trastuzumab Emtansine	Pertuzumab	Lapatinib	Neratinib
			Confers increased sensitivity	Confers increased sensitivity	Confers increased sensitivity	Confers increased sensitivity	Confers increased sensitivity
Thymic Malignancies	KIT E490K	Extra-cellular domain [exon 9]	Imatinib	Sunitinib	Sorafenib	Dasatinib	
			May confer increased sensitivity	May confer increased sensitivity	May confer increased sensitivity	May confer increased sensitivity	
	KIT Y553N	Juxtamembrane domain [exon 11]	Confers sensitivity	Unknown at this time	Unknown at this time	Unknown at this time	
	KIT W557R	Juxtamembrane domain [exon 11]	May confer increased sensitivity	May confer increased sensitivity	May confer increased sensitivity	May confer increased sensitivity	
	KIT V560	Juxtamembrane domain [exon 11]	Confers increased sensitivity	May confer increased sensitivity	Unknown at this time	May confer increased sensitivity	

	KIT P577_ D57 9del	Juxtamembrane domain [exon 11]	Unknown at this time	Unknown at this time	Confers sensitivity	Unknown at this time
	KIT D820E	Kinase domain [exon 17]	May confer decreased sensitivity	May confer decreased sensitivity	Confers increased sensitivity	May confer increased sensitivity

Gastrointestinal Stromal Tumor (GIST)	BRAF	V600E mutation substitution at position 600 in BRAF Kinase domain [exon 15]	Imatinib	Sunitinib
			Confers resistance	Confers resistance
	KIT	Extracellular dimerization motif [Exon 9]	Confers intermediate sensitivity; Higher doses of imatinib [up to 800 mg total daily dose] may be more effective in metastatic disease than 400 mg oral daily	Confers increased sensitivity
		Juxtamembrane domain [Exon 11]	Confers increased sensitivity	Decreased sensitivity to second-line sunitinib; Too few patients have been treated in the imatinib-naïve setting
		Tyrosine kinase 1 [TK1] domain/ATP binding pocket [Exon 13]	Confers sensitivity as a primary mutation; Confers resistance as a secondary mutation	Sensitive in in vitro studies; Too few patients in imatinib naïve or second-line settings to determine
		Tyrosine kinase 1 [TK1] domain/ATP binding pocket [Exon 14]	Confers resistance as a secondary mutation	Sensitive in vitro studies; Too few patients in imatinib naïve or second-line settings to determine
		Tyrosine kinase 2 [TK2] domain and activation loop [Exon 17]	Primary mutation sensitive in vitro; Confers resistance as a secondary mutation	Confers resistance as secondary mutation; Too few people treated in imatinib- naïve setting to determine activity
	PDGFRA D842V	Tyrosine kinase 2 [TK2] domain [Exon 18]	Confers decreased sensitivity	Confers decreased sensitivity
Bladder Cancer	TSC1 E636fs	frameshift introduction of stop codon into the TSC1 gene [Exon 15]	mTOR inhibitors: Confers increased sensitivity	
Inflammatory myofibroblastic tumor (IMT)	ALK Fusions	Chromosomal rearrangements involving the ALK gene on 2p23	ALK inhibitors: Confers increased sensitivity	
Gastric Cancer	HER2	HER2 amplification	Trastuzumab: Confers increased sensitivity	

Non-Small Cell Lung Cancer (NSCLC)	ALK Fusions	Chromosomal rearrangements involving the ALK gene on 2p23	ALK TKIs	HSP90 inhibitors	EGFR TKIs
			Confers increased sensitivity	Confers increased sensitivity	Confers decreased sensitivity
	EGFR	Kinase domain [exon 19] Insertion	Unknown at this time	Unknown at this time	Confer s increased sensitivity
		Kinase domain [exon 19] DELETION	Unknown at this time	Unknown at this time	Confers increased sensitivity
		Kinase domain [exon 20] Insertion	Unknown at this time	Unknown at this time	Confers decreased sensitivity
	ROS1 Fusions	Chromosomal rearrangements involving the ROS1 gene on 6q22	Crizotinib		Erlotinib/Gefitinib
			Confers increased sensitivity		Confers decreased sensitivity

Table 3: Examining the effect of mutations in more detail by looking at the locations of mutations in different types of cancers and the effects of different anticancer drugs. *data has been taken from mycancergenome.com

When using breast cancer as an example, upon comparing the number of mutations (111 mutations) with the number of sequenced genes (36 genes), the results show that there is an absolute need to detect all of the mutations and to use the given information to find the best tools and methods in diagnosis and treatment (Table 3).

For example, in chronic myelogenous leukemia [CML], which is a type of cancer that affects humans, the presence of BCR-ABL1 fusions at the location t [9;22] [q34.1;q11.21] confers sensitivity to 5 anticancer drugs, whereas the presence of the BCR-ABL1 Y253H mutation in the P-loop region of the kinase domain [exon 4] confers reduced sensitivity to imatinib and nilotinib but retains sensitivity to dasatinib. The effects of ponatinib and bosutinib are unknown. Additional studies and clinical trials are still required to understand their effects.

Targeted cancer therapy

Above, we describe how mutations in genes will affect the outcome of therapy and move the science of drug design forward. In cancer research, the development of drugs that target carcinogenesis events is called targeted cancer therapy or rational drug design. The primary goal of these targeted drugs is to interfere with specific molecules involved in cancer without causing any harm to normal cells. Targeted drugs are the cornerstone of precision medicine and the current focus of anticancer discovery and development [38].

Standard chemotherapy works in a non-selective manner because it acts on all rapidly dividing cells, whether they are cancerous or normal, but targeted cancer therapies are designed and synthesized to be delivered to and only react with their target; therefore, they act on specific molecular targets that are associated with cancer. In cytogenetics, targeted therapies are cytostatic [they block tumor cell proliferation], whereas standard chemotherapy agents are cytotoxic [they kill tumor cells] [38].

With the recent advances in genetic medicine and the sequencing of the human genome, the concept of a "druggable genome" has become more applicable and popular [39-41]. A number of approaches are used to identify potential targets, and it is always possible to discover new approaches. One approach is to compare the amount of proteins present in cancer cells with the amount of proteins present in normal cells. An example of a differentially expressed target is the human epidermal growth factor receptor 2 protein [HER-2], which is expressed at high levels in some cancer cells.

Several targeted therapies are directed against HER-2, including trastuzumab [Herceptin®] [38]. In addition, non-small cell lung cancer sequencing has shown the presence of EGFR tyrosine kinase inhibitor sensitivity resistance, which has resulted in the use of routine EGFR sequencing for therapeutic purposes [34]. Recently, a study was published online (23 September 2014) that discussed the use of Mir-34 as a new weapon against cancer [42].

Another approach is to determine whether cancer cells produce mutant [altered] proteins that drive cancer progression. For example, BRAF, which is a cell growth signaling protein, has been found to be present in an altered form [BRAF V600E] in many melanomas. The current approved drug is vemurafenib [Zelboraf®], and it targets the mutant form of the BRAF protein to treat patients with this type of cancer (Table 4) [38].

The primary methods of targeted cancer therapy include hormone therapies, signal transduction inhibitors, gene expression modulators, apoptosis inducers, angiogenesis inhibitors, immunotherapies, and toxin delivery molecules [38]. Hormone therapies, which are used to treat tumors that are sensitive to hormones, such as breast cancer, uterine cancer and prostate cancer, work by reducing the hormones that stimulate tumor growth or by blocking the hormone receptor. For example, aromatase inhibitors, such as anastrozole, block the formation of estrogen, tamoxifen citrate blocks antiestrogens, bicalutamide blocks androgen receptor antagonists, and abarelix [antagonist] and gosrelin [analog] block GnRH analogs and its antagonists [43].

Kinase inhibitors, which are a new group of anticancers known as signal transduction inhibitors, target the cellular mechanisms of signal transduction and block the activity of molecules that play a role in or participate in these mechanisms. Examples include imatinib, which was the first agent in this group and works as a BCR-ABL kinase inhibitor; dasatinib, which is a multikinase inhibitor; everolimus, which is an mTOR inhibitor; and gefitinib, which is an EGFR tyrosine kinase inhibitor [43].

Gene expression modulators modify the functions of proteins that play a role in controlling gene expression. For example, triplex-forming oligonucleotides [TFO] bind to the major groove of duplex DNA, leading to the creation of a third strand or a triple helix [44,45].

Apoptosis inducers are also used because cancer cells avoid apoptosis. Therefore, the mechanism of action of this group is strategies that result in the death of cancer cells [38].

Angiogenesis inhibitors block the nourishment of the tumor by blocking the growth of new blood vessels or by targeting the molecules responsible for stimulating the growth of new blood vessels. For example, itraconazole inhibits VEGFR phosphorylation [46,47].

Monoclonal antibodies, which are designed to target the proteins expressed in tumor cells, make the cancer cell more visible to the immune system. For example, rituximab, which targets CD20, blocks growth signals; cetuximab, which targets EGFR, stops new blood vessels from forming; bevacizumab, which targets VEGFR, delivers radiation to cancer cells; and ibritumomabtiuxetan, which is labeled with yttrium 90, blocks CD20 [43].

FDA-Approved Indication[S]	Target[S]	Targeted Drugs
Colorectal cancer [KRAS wild type]	EGFR [HER1/ERBB1]	Panitumumab [Vectibix]
Renal cell carcinoma	VEGFR, PDGFR, KIT	Pazopanib [Votrient]
Melanoma	PD-1	Pembrolizumab [Keytruda]
Breast cancer [HER2+]	HER2 [ERBB2/neu]	Pertuzumab [Perjeta]
Chronic myelogenous leukemia Acute lymphoblastic leukemia [Philadelphia chromosome positive]	ABL, FGFR1-3, FLT3, VEGFR2	Ponatinib [Iclusig]
Gastric cancer or Gastroesophageal junction [GEJ] adenocarcinoma	VEGFR2	Ramucirumab[Cyramza]
Colorectal cancer Gastrointestinal stromal tumors	KIT, PDGFRβ, RAF, RET, VEGFR1/2/3	Regorafenib[Stivarga]
Non-Hodgkin's lymphoma Chronic lymphocytic leukemia Rheumatoid arthritis Granulomatosis with polyangiitis	CD20	Rituximab [Rituxan, Mabthera]
Cutaneous T-cell lymphoma Peripheral T-cell lymphoma	HDAC	Romidepsin[Istodax]
Myelofibrosis	JAK1/2	Ruxolitinib[Jakafi]
MulticentricCastleman's disease	IL-6	Siltuximab[Sylvant]
Hepatocellular carcinoma Renal cell carcinoma Thyroid carcinoma	VEGFR, PDGFR, KIT, RAF	Sorafenib[Nexavar]
Renal cell carcinoma	mTOR	Temsirolimus[Torisel]
Rheumatoid arthritis Juvenile idiopathic arthritis	IL-6R	Tocilizumab[Actemra]
Rheumatoid arthritis	JAK3	Tofacitinib[Xeljanz]
Non-Hodgkin's lymphoma	CD20	Tositumomab[Bexxar]

Melanoma [with BRAF V600 mutation]	MEK	Trametinib[Mekinist]
Breast cancer [HER2+] Gastric cancer [HER2+]	HER2 [ERBB2/neu]	Trastuzumab[Herceptin]
Medullary thyroid cancer	EGFR [HER1/ERBB1], RET, VEGFR2	Vandetanib[Caprelsa]
Melanoma [with BRAF V600 mutation]	BRAF	Vemurafenib[Zelboraf]
Basal cell carcinoma	PTCH, Smoothened	Vismodegib[Erivedge]
Cutaneous T-cell lymphoma	HDAC	Vorinostat[Zolinza]
Colorectal cancer	PIGF, VEGFA/B	Ziv-aflibercept[Zaltrap]

Table 4: Targeted therapies that have been approved by the FDA.

Precision medicine

Personalized medicine, genomic medicine, stratified medicine, and targeted medicine are concepts that focus on characterizing individual biological profiles by analyzing the genome and using the results to guide medical decisions to provide patients with the best health care. Precision medicine is generally defined as providing the right patient with the right drug at the right time by precisely targeting the molecular pathways that caused the disease. Precision medicine links the multifactorial characteristics of each individual. A unique feature of precision medicine is that it does not depend only on genomic medicine; it also incorporates a patient's lifestyle, non-genomic biological information, environmental parameters and other relevant data [1].

NGS, which has paved the way for precision medicine, has gained many successes in the field of oncology, allowing each individual to have a comprehensive genomic profile. This provides many advantages for the cancer patient. For example, it allows the generation of specific tumor data based on the patient's characteristics, which can then be used later for targeted therapy, genome-based blood tests, biomarkers and other clinical applications.

Roy Chowdhury's report was the first study to apply NGS in personalized oncology. He showed that using such techniques not only improved patient health care but also made the best use of the existing available genetic information. The aim of the study was to discover the practical challenges of using NGS in the field of clinical oncology. The researchers implemented whole-genome sequencing [WGS], targeted whole-exome sequencing [WES] [the coding regions of the genome] of both normal and cancer DNA, and transcriptome sequencing [RNA-seq] [transcribed RNAs] for each patient [48].

The results detected many mutation categories [gene expression alterations, structural rearrangements, point mutations, copy number alternations], and the findings were discussed by a multidisciplinary sequencing tumor board [STB], which included a panel of a wide range of specialists, including those working in clinical oncology, cancer genetics, bioinformatics, clinical pathology, social and behavioral sciences and bioethics [49].

Two patients were enrolled in the pilot study, one with melanoma and the other with colorectal cancer, and both were treated unsuccessfully. After determining the final results of their sequencing and taking a deep look into their genetic profiles, the melanoma patient was shown to have HRAS, a CDKN2C structural rearrangement. The sequencing tumor board [STB] suggested using PI3K and MEK inhibitors as a treatment for the patient. Sequencing results in the colorectal cancer patient detected CDK8, an NRAS variation that may be used as a future therapeutic target [48].

Additionally, one of the most successful cases of the use of NGS in clinical practice is an inspiring study that was performed at Washington University and was headed by genetics researchers who performed transcriptome sequencing and whole-genome sequencing [WGS] for a researcher on the team affected by adult acute lymphoblastic leukemia [ALL]. The results showed that the FLT3 gene was highly active in the leukemia cells. The anticancer drug sunitinib, which is approved by the FDA to treat gastrointestinal stromal tumors, renal cell carcinoma, and pancreatic cancer, inhibits FLT3. After using sunitinib, the patient's blood returned to normal (Table 5) [50-60].

Biopharmaceutics

One of the major classes of anticancer agents are monoclonal antibodies, which bind to a target and activate the host's immune mechanisms, leading to the killing of the cancer cells by either complete mediated lysis or via killer cells. Monoclonal antibodies can attach to an inactive growth factor receptor on a cancer cell, thus inhibiting survival pathways and promoting apoptosis. Cetuximab is a monoclonal antibody directed against EGFR, erbitux is a well-known anti-EGFR antibody, rituximab lyses B lymphocytes by binding to the calcium channel forming CD20, and trastuzumab binds to HER2.

The importance of NGS in this field lies in generating an antibody library because NGS data can provide a more precise analysis of multiplicity in an antibody library [61]. In addition, NGS can be used in interactome profiling to detect protein interactions with multifunctional enzymes [62], thus providing new potential methods for studying protein-protein interactions and antibody-antigen binding [61].

Polypharmacology

The philosophy of drug design has been converted to the idea of one drug multiple targets rather one drug one target [63-68]. Polypharmacology is still a major challenge in drug development. This technique includes using a single drug that is capable of affecting multiple targets in a specific disease pathway or using a single drug that affects multiple targets connected to multiple disease pathways [69]. Polypharmacology aims to explore drug repurposing, which includes exploring the unidentified off targets of available drugs [70-72]. In regards to anticancers, polypharmacology is considered a primary treatment approach because anticancers are often taken in combination with MOPP, a curative combination prototype that consists of nitrogen mustard, vincristine [Oncovin], procarbazine and prednisone.

The importance of NGS lies in using this approach for targeting, to increase a drug's efficacy and minimize its toxicity. Additionally, NGS can detect a wide variety of rare, actionable mutations that can be used as targets, and polypharmacology aims to find multitargets; therefore, they are complementary to each other.

NGS provides multitargets, and polypharmacology delivers the drug. In addition, NGS can be used for ligand-based predictions, target-based predictions, and phenotype-based predictions [including

Cancer Type	Sample	Major Findings	Findings Importance
Whole Genome sequencing (WGS)			
Melanoma	WGS:25	Detect : - Mutated gene, PREX2.-Comprehensive sequencing of human melanoma tumors.	PREX2 mutations are biologically related , ectopic expression of mutant PREX2 accelerated tumor formation of immortalized human melanocytes in vivo [52].
Breast cancer	WES :103 WGS:22	Detect: - Repeated mutation in CBFB.-Deletion of RUNX1. -Repeated MAGI3-AKT3 fusion.	MAGI3-AKT3fusion led to AKT kinase activation which cancelled by ATP-competitive AKT small molecular inhibitor treatment [53].
Whole exome sequencing (WES)			
Uterine serous carcinoma	WES:10	Detect : Somatic mutation in TP53, PIK3CA, FBXW7, PPP2R1A.	Cyclin E- FBXW7, PI3K , TP53 have a role in the etiology of uterine serous carcinoma [54].
Head and neck squamous cancer	WES:32	Detect :- Mutation in FBXW7, NOTCH1.	40% of 28 identified mutations in NOTCH1 were truncate gene product. - NOTCH1 may function as tumor suppressor [55].
Transcriptomic sequencing			
Colon cancer	WGS:2 WES:72 RNA-seq:68	Detect :- IGF2 over expression. - Repeated gene fusion RSPO2,RSPO3 .	-RSPO may have a role in Wnt signaling activation and tumerigenesis . -R spondin gene fusion provide new therapeutic opportunities [56].
thyroid cancer cells [TPC-1] and leukemia cells	MicroRNAs:90	Defined region upstream of a conserved GXGXXG kinase motif.	* Tyrosine kinase [TK] fusions are attractive drug targets in cancers * identified for the first time the genomic fusion sequences of CCDC6-RET in TPC-1 cells and FGFR1OP2-FGFR1 in KG-1 cells [57].
Targeted sequencing			
familial breast cancer	N=12	-Identified all 19 distinct BRCA1 and 35 distinct BRCA2 sequence alterations. *Detection of BRCA1 , BRCA2 variants from introns and untranslated regions.	Individuals and families carrying mutations in BRCA1 and BRCA2 [BRCA1/2] have a markedly elevated risk of developing breast and ovarian cancers [58].
Prostate cancer	N = 45	-182 cancer associated genes. -37 introns of commonly rearranged genes. -Novel and actionable BRAF rearranged.	Designing targeted assays to detect driving mutations, drug targets , future therapies, biomarkers [59].
Epigenetic sequencing			
Bladder cancer	N = 212	- 1,627 hypermethylated promoter targets in the BC cell. - VAX1 , LMX1A are associated with BC recurrence. - VAX1, KCNV1, TAL1, PPOX1, and CFTR are associated with BC diagnosis.	Identified a promising diagnostic marker panel for early non-invasive detection and subsequent BC surveillance [60].
Non-small cell lung Cancer	N = 48	- Identified 57 differentially methylated regions [DMRs] present in all NSCLC tumors. - Hypermethylated DMRs were strongly associated with genes encoding transcriptional regulators. - Subtelomeric regions and satellite repeats were hypomethylated in the NSCLC samples. - Identified DMRs that were specific to adenocarcinomas and squamous cell carcinomas.	Provide a resource containing genome-wide DNA methylation maps of NSCLC and their paired lung tissues, and comprehensive lists of known and novel DMRs and associated genes in NSCLC [61].

Table 5: Studies showing the remarkable success of using NGS in precision medicine

transcriptomic-based methods, proteomics-based approaches, and drug target identification [73].

Toxgnostics

Most anticancers are toxic and cause severe side effects, such as cisplatin, which causes nephrotoxicity and ototoxicity; temozolomide, which causes genotoxicity, fetotoxicity, and teratogenicity; and 6-mercaptopurine, which is dose-dependent and causes hepatotoxicity. These side effects have led to the field of toxgnostics in cancer medicine. Toxgnostics, which is generally defined as a systemic study, focuses on studying genetic toxicity predictors that are related to and caused by anticancers [74].

NGS revolutionized our ability to predict anticancer toxicity, determine the causes of toxicity and detect toxgnostic variants. NGS can also predict the toxicity level in each patient based on the genetic profile and the number of people who may be responsive to a specific drug. Toxgnostics aims to find the relationship between a plasma drug concentration profile and the toxicity level and to create a personalized treatment plan for each patient, which may require elective dose adjustment for a select drug [74].

Vaccinology

NGS can be utilized in the initial development stages 3 of a vaccine to evaluate its efficiency and safety [75]. Due to the accuracy of NGS and its ability to detect sequence variants in each individual, NGS can provide more reasons and solutions to help understand why some people may face side effects after receiving vaccines, but others may not [61].

Pharmacoepidemiology

Pharmacoepidemiology, which uses variations in study designs to determine the effects of drug treatments on populations and clinical situations [76]. NGS has been successfully used in this field because epigenetics plays a role in tumorigenesis and paves the way for drug discovery. The two important mechanisms of epigenetics are DNA methylation and histone modification. For example, epigenetics can be used for the following:

-epigenetic-based biomarkers [for colorectal cancer, septin 9 is used to block methylation] [77].

-epigenetic-based drugs [for treatment of cutaneous T-cell lymphoma, vorinostat, a histone deacetylase inhibitor from Merck, is used [78,79].

Conclusion

Next-generation sequencing has proven to be the cornerstone of advanced research. This includes not only cancer research but also all areas of human genetics. By improving the speed and precision of the results, designing new therapeutic strategies can be achieved to improve the outcome of cancer treatments and save as many lives as possible.

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