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Chapter

Microarrays and NGS for Drug Discovery

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Abstract

Novel technologies and state of the art platforms developed and launched over the last two decades such as microarrays, next-generation sequencing, and droplet PCR have provided the medical field many opportunities to generate and analyze big data from the human genome, particularly of genomes altered by different diseases like cancer, cardiovascular, diabetes and obesity. This knowledge further serves for either new drug discovery or drug repositioning. Designing drugs for specific mutations and genotypes will dramatically modify a patient's response to treatment. Among other altered mechanisms, drug resistance is of concern, particularly when there is no response to cancer therapy. Once these new platforms for omics data are in place, available information will be used to pursue precision medicine and to establish new therapeutic guidelines. Target identification for new drugs is necessary, and it is of great benefit for critical cases where no alternatives are available. While mutational status is of highest importance as some mutations can be pathogenic, screening of known compounds in different preclinical models offer new and quick strategies to find alternative frameworks for treating more diseases with limited therapeutic options.

Keywords: NGS, microarray, transcriptomics, drug discovery

1. Introduction

Over the last few decades, major breakthroughs in scientific and technology-related fields have been made, contributing to major gains and significant advances in clinical practices in dealing with cancer diagnosis, treatment, and preventive measures. Although we are now better informed, skilled, and equipped than ever before, efforts for administering effective cancer treatments, and drugs do not appear to have advanced far enough. In spite of all the changes implemented in translational oncology due to availability of new and sophisticated molecular tools, there is a missing link between pre-clinical data and actual findings. Although significant funds have been allocated for pre-clinical studies, 95% of therapeutic strategies have not passed phase I clinical trials in humans. It is likely that those settings prior to drug development may not be adequate enough to effectively mimic human responses. Those few drugs that are approved by regulatory agencies have had either very little or no effects on overall survival rates. Therefore, the lack of efficacy associated with

the current anticancer drugs along with the high treatment costs are both contributing to growing incidence and mortality of cancer patients worldwide [1–3].

Cancer, the umbrella term used for a series of more than 200 different neoplastic diseases caused by abnormal cellular divisions due to either singular or cumulative genomic events is, without a doubt, the most dreaded health problem over the past centuries, including current times [4]. According to GLOBOCAN, cancer is one of the leading causes of death, accounting for more than 18 million cases worldwide. These cases are expected to increase by approximately 70% over the next two decades [5–7]. As the incidence of cancer continues to grow, and the disease becomes more difficult to treat, the challenge of discovering new and more effective anticancer drugs is more critical than ever before [8].

From a historical point of view, secondary metabolites extracted from different natural products were the very first known sources of new therapeutic compounds. Early on, the screening process of every novel drug was rather simple, usually based on various ethnobotanical claims, and often fueled by serendipity [9, 10]. However, this traditional approach was soon replaced with modern methods.

With the advent of modern omics technologies, and ever-expanding knowledge of the human genome, as well as of genomes of various organisms, including pathogenic ones, drug discovery has evolved into a therapeutic target-based approach. Moreover, recent computational advances in handling and analysis of big data, particularly of complex biological information, have become more user-friendly and less time-consuming. All these developments have accelerated the process, and have paved the way for the beginning of the modern drug discovery era [9, 11].

Early pioneering discoveries of Claude Bernard, Louis Pasteur, and Robert Koch, followed by significant findings in other disciplines, such as in organic chemistry, have set many milestones by the end of the nineteenth century. These developments have laid the foundations for what it is know as the era of modern drug discovery, one of the most provocative scientific fields. Since then, myriad treatments have become available, and many diseases, including those of viral, bacterial, and parasitic infections, as well as of diabetes and cardiovascular disorders, along with various types of cancer have become either treatable, curable, or at least can be held off at symptomatic levels. Furthermore, modern drug discovery has aided in the identification of several pharmacological compounds that would promote safety of many surgical procedures, and have contributed to successful cell- and solid- organ transplantation [9, 12].

Drug discovery is a very long, challenging, and complex multistep process that can be generally split into four main steps, as follows: target selection and validation; compound screening and lead candidate optimization; preclinical studies; and clinical trials [11]. Although it is highly desirable to develop a rapid and effective treatment for every disease, drug development remains a lengthy process, requiring up to 15 years of work along with millions to billions of dollars simply to turn a single drug candidate into an efficient, safe, and accessible product. In particular, costs for cancer care in the United States are projected to reach up to $246 billion by 2030 (a 34% increase from 2015), while anticancer drug development remains at a high rate of failure [13–15].

Over the past 25 years, benefits of implementing many innovative scientific technologies have been made possible due to advances in molecular research studies prior to anticancer drug discovery [16]. Further, our understanding of cancer biology has significantly expanded, as new molecular strategies have exceeded the expectations, and helped rising the overall patient survival rates [17, 18]. Furthermore, as academic research centers have begun to openly embrace collaborations with pharmaceutical and biotechnology companies, the ecosystem for drug discovery has become more responsive and efficient than ever before. As a result, there have

been vast expansions of the chemical compound libraries, well beyond those known natural products that have been exploited in the past. Modern technologies, such as high-throughput screening (HTS), fragment-based screening (FBS), molecular modeling, crystallography, nanotechnology, and advanced chemistry, among others, are also currently playing important roles in the revolution of drug discovery [9, 19].

Next generation sequencing (NGS), also known as massively parallel sequencing, refers to a number of molecular high-throughput methods that follow the same principle of simultaneously deciphering millions of nucleotide sequences in a fast, accurate, and affordable approach. Unlike previous sequencing technologies, a whole genome can be sequenced at once, producing 100-folds more data than other tools based on Sanger's sequencing method. Thus, NGS has become one of the major omics technologies to be adopted in life sciences fields, including functional genomics, metagenomics, transcriptomics, and oncogenomics. Therefore, HTS methods, such as those of the NGS repertory, are expected to notably accelerate drug discovery and reduce associated costs [20, 21].

Another omics technology of interest is that of microarrays, as this tool allows for simultaneous analysis of DNA and assessing of mRNA expression levels. As a result, microarrays have been rapidly exploited in various research studies, including those focused on drug discovery, as they afford a better understanding of both the pathological mechanism and drug activity. Furthermore, microarray technologies are useful in identifying a drug target or a biological compound(s) that may interact with a synthetically designed drug. In addition, the microarray technology is highly efficient and of low-cost, but has some limitations, particularly pertaining to availability of advanced bioinformatic analysis tools [22]. These two omics technologies will be further discussed below.

Overall, the above-mentioned technologies and tools will have major impacts on revolutionizing drug discovery efforts, including identifying more efficacious and effective anticancer drugs in shorter periods of time, and at reduced costs [23].

2. Microarrays and drug discovery

### 2.1 Microarray technology: role, types, and applications

The microarray technology is powerful though early on it has had some limitations due to its high costs. However, in recent years, it has become more affordable with the availability of commercial microarray chips and platforms. Thus, this technology has moved from research laboratories to clinical applications. In recent years, microarrays have played significant roles in drug discovery. A large number of studies have demonstrated that microarray datasets not only allow for rapid and direct analysis of large amounts of biological information, but these also promote identification of potential biomarkers for various diseases [24–28]. Furthermore, microarray datasets can potentially determine the appropriate drug dose that can maximize its therapeutic effect. In clinical trials, microarrays can be used for early detection of any toxicity or any side-effects of a drug or a drug dose in order to provide rapid, sensitive, and safe treatments. Moreover, microarrays play important roles in pharmacogenomics by allowing for identification of associations between responses to drug treatment and a patient's genetic profile [28, 29], as well as for selecting the most appropriate new candidate drugs for clinical trials.

There are several types of microarrays, including DNA microarrays, microRNA arrays, chemical compound microarrays, antibody microarrays, protein microarrays, tissue microarrays, and carbohydrate arrays. In clinical research, DNA microarrays are often used for novel biomarker discovery [30]. Among other applications

of DNA/RNA microarrays are the following: 1) identification of differential gene expression, 2) analysis of mutations, 3) screening of single nucleotide polymorphisms (SNPs), 4) determination of methylation, acetylation, and alternative splicing, and 5) comparative genomic hybridization [31–34].

Microarrays consist of hundreds to thousands of DNA, RNA, oligonucleotides, or other probe molecules that are immobilized in an array format onto a solid support surface, such as microscope glass slides, silicon chips, or nylon membranes, and then exposed to labeled samples carrying corresponding target molecules to allow for simultaneous detection of nucleic acid/protein/antibody/other targets. Typically, a single probe is at one-time leading to a microarray with hundreds of thousands of different oligonucleotide sequences complementary to distinct fragments of known DNA or RNA sequences [35]. Components of a DNA or an RNA sample loaded onto a slide/chip/membrane will hybridize specifically to their complementary probes, and the fluorescence intensity will correspond to the amount of DNA or RNA of a given gene in a sample [36].

Microarrays are processed in either "one-color" or "two-color" formats. In a one-color format, a single RNA sample is labeled with a fluorophore, such as cyanine-3 (Cy-3) or cyanine-5 (Cy-5) prior to hybridization, and the intensity of the fluorophore is determined [37]. Whereas a two-color microarray capitalizes on a competitive hybridization (**Figure 1**). In this format, a single nucleic acid sample is labeled with a green dye, while a related sample is labeled with a red dye. Following hybridization and removal of unbound nucleic acids, a laser scanner will detect those red- and green-labeled molecules. The intensity of each colored spot on an array is determined, and the red/green ratio is determined [38].
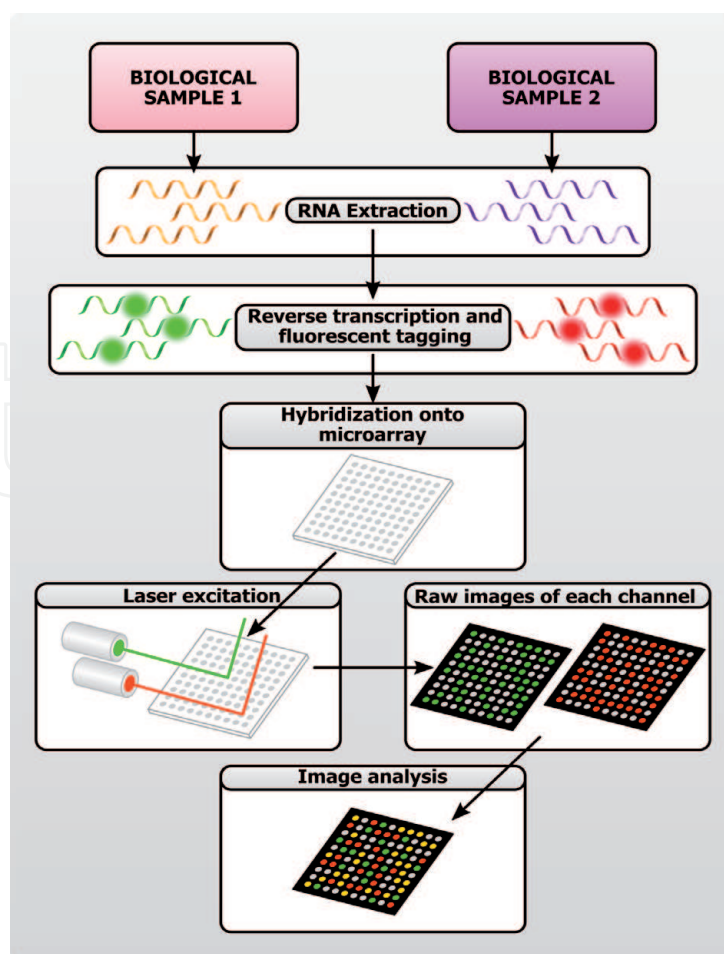
Figure 1.
A general workflow for a typical two-color microarray experiment.

Several microarray technologies have been developed using various platforms that have been optimized to maximize reproducibility and accuracy of findings [39]. For example, Affymetrix GeneChip microarrays are manufactured using photolithography that utilize oligonucleotide probes. This system has the capability of monitoring expression of every gene in a genome. In fact, Affymetrix GeneChips have been used for genotyping, copy number analysis, transcriptome analysis, and miRNA profiling. On the other hand, Agilent oligonucleotide microarrays are based on inkjet technology for *in situ* manufacturing of probes, wherein actual probe sequences are used as linkers in order to extend these probes to provide higher specificity [39]. Whereas, Illumina BeadArrays are based on patterned substrates for high-density detection of target nucleic acids using silica microbeads [40].

Some of the common available techniques used in drug development efforts, including microarrays, are listed in **Table 1**.

2.2 Droplet Digital PCR (ddPCR) and microarrays

The Droplet Digital PCR (ddPCR) is a recent technology that is commercially available, capitalizing on the use of *Taq* polymerase in a standard PCR reaction in order to amplify a target DNA fragment from a complex sample using pre-validated primers or primer/probe assays [61, 62]. Galbiati et al. have proposed a workflow that combined a microarray assay with ddPCR for both detection and quantification of circulating tumor DNA mutations in colon cancer patients [63]. This approach is useful for the development of reliable non-invasive biomarkers for RAS and BRAF mutations, identifying a target mutation, and providing clinically relevant information. Microarray analysis and ddPCR data have identified mutations in primary breast tumors from female patients treated with adjuvant mono-tamoxifen therapy [64]. Moreover, using microarray and ddPCR, it is observed that epidermal growth factor receptor (EGFR) expression can be used as a prognostic biomarker in patients with oropharyngeal squamous cell carcinoma, as it is associated with smoking status [65]. In another study, microarray analysis of uterine tissue, along with validation using ddPCR has allowed for observing downregulation of genes in pathways of

Techniques	Applications	References
ChIP microarrays	Drug development Pharmacogenomics Gene discovery Gene expression profiling	[41–46]
Splice variants	Pharmacogenomics Drug discovery Biomarker identification Polymorphism/SNP detection Drug target identification	[47–52]
Genotyping	Drug discovery Pharmacogenomics Environmental monitoring Drug resistance Vaccine candidate identification	[53–57]
Comparative genomic hybridization (CGH)	Gene discovery Biomarker identification Clinical application Vaccine candidate identification	[29, 52, 58–60]

Table 1.
Available techniques for drug discovery.

the immune response following tetrabromobisphenol A treatment [66]. Moreover, ddPCR analysis of miRNAs identified using a microarray assay has revealed that anti-apoptotic miRNA may be potentially involved in antagonistic effects between the *Alternaria* mycotoxins alternariol and altertoxin *II* in HepG2 cells [67]. In another study using this combined approach, transglutaminase 2 is identified as a novel regulator of the tumor microenvironment in gastric cancer patients, thus serving as a promising target for restricting tumor-promoting inflammation [68].

2.3 Undruggable to druggable proteins using microarrays

In recent years, efforts have been directed towards transforming those proteins that are deemed pharmacologically incapable of being targeted, coined as "undruggable", into "druggable" proteins. Despite the fact that many proteins, such as kinases, that promote cancer development, are capable of serving as drug targets, proteins such as RAS, MYC, and p53 are deemed as "undruggable targets" [69]. Thus, overcoming these "undruggable targets" becomes one of the main challenges for drug discovery. One of the new proposed methods to overcome these challenges is represented by the inhibition of kinase activities of oncogenic proteins using small molecules and antibodies [70]. In one approach, blocking of pathways downstream of a target protein has served as a viable strategy to assess the functional role of a mutation as an oncogenic driver of different types of cancers, and for serving as a valid clinical trial design [71]. In another approach, discovery of hidden allosteric sites is an effective strategy for development of new drug targets, as well as for discovery of allosteric drugs [69].

It is known that *RAS* mutations serve as early genetic events in tumor progression, while sustained expression of *RAS* mutations are deemed necessary for tumor maintenance [72]. Although RAS have been deemed as "undruggable", recent studies have demonstrated that therapies targeting either RAS-activating pathways or RAS effectors pathways combined with direct RAS inhibitors, along with immune checkpoint inhibitors or T-cell targeting methods, *RAS*-mutant tumors are found to be treatable [73]. As the transcription factor MYC promotes cancer progression, small-molecule inhibitors are used to drug the "undruggable" by inducing epigenetic silencing and regulating G-quadruplex structures within the *MYC* promoter [74]. In another example, *p53* is well known as the most frequently altered gene in human cancer, and therefore the p53 mutant protein is deemed as an important undruggable target [75]. Such compounds as p53 reactivation, induction of massive apoptosis-1 (PRIMA-1), and a structural analogue of PRIMA-1, APR-246, have been found to reactivate the mutant p53 protein by converting it to a form with wild-type properties [75].

Using a custom-designed lncRNA microarray, Orilnc1 was identified as a novel nonprotein mediator of RAS/RAF activation, with potential applications as a therapeutic target in RAS/RAF-driven cancers [76]. An Affymetrix microarray revealed coexpression of a mutant β-catenin and K-Ras in mice by targeting β-catenin in hepatocellular cancers [77]. Microarray and pathway enrichment analyses revealed that MYC expression could be downregulated by 1,2,3,4,6-penta-O-galloyl-beta-D-glucopyranoside (PGG) in hepatocellular carcinoma [78]. Using a genome-wide microarray analysis, it was reported that targeting c-Myc would unlock novel strategies to combat asthma [79]. β-catenin could be deemed as an anticancer therapeutic target by regulating c-Myc and CDKN1A expression in breast cancer cells [80]. In addition, microarray data identified and characterized novel p53 target genes expressed in hepatocarcinoma cells, and were associated with steroid hormones processing and transfer [81]. Furthermore, it was proposed that there was a novel non-cell-autonomous tumor-suppressive regulation, mediated by p53, playing a key role in maintaining organism homeostasis. Moreover, breast cancer

metastasis suppressor 1-like (BRMS1L) was found to be upregulated by p53 protein. In addition, p53 inhibited cancer cell invasion and migration, and thus could serve as a therapeutic target for cancer [82].

2.4 Microarrays and drug resistance

Resistance to chemotherapy remains a major obstacle to improving a cancer patient's outcome and survival despite significant advances in surgery, radiation therapy, and anticancer treatments. In cancer, drug resistance arises from a complex range of molecular and biochemical processes, such as modifications in DNA repair mechanism, drug uptake, absorption, and metabolism. Recent studies have identified two forms of drug resistance in cancer patients, intrinsic (innate resistance that is present before a patient is exposed to drugs) and acquired (a direct result of chemotherapy). A growing number of microarray studies have exploited the identification of mechanisms involved in both drug response and drug resistance in clinical samples in order to identify biomarkers for drug resistance [83]. For example, microarray analysis has provided a better understanding of circular RNA expression profiles that are associated with gemcitabine resistance in pancreatic cancer cells [84]. In human gastric cancer tissues, a microarray study has revealed that miR-424 regulates cisplatin resistance of gastric cancer [85]. Furthermore, extracellular matrix proteins have been implicated in drug-resistant ovarian cancer cells, thus inhibiting penetration of a drug into cells, as well as contributing to increased apoptosis resistance [86].

Of particular interest, new genes associated with drug resistance development in ovarian cancer have been discovered using microarray analysis, wherein 13 genes are found to be upregulated, while nine genes are found to be downregulated [87]. In triple-negative breast cancer cells, notable alterations are observed at both transcriptomic and genomic levels, along with identification of a mutation (*TP53*) associated with drug response [88]. In another study, bioinformatics analyses of microarray datasets have identified neuromedin U (NMU) as a potential gene that confers alectinib resistance in non-small cell lung cancer [89]. Furthermore, expression profiling has allowed for discovery of genes involved in ovarian-drug resistance, wherein these genes are found to be controlled via different signaling pathways, including MAPK–Akt, Wnt, and Notch [90]. In another study, microarray analysis has found that tumor initiation and insulin-like growth factor (IGF)/fibroblast growth factor (FGF) signaling contribute to sorafenib resistance in hepatocellular carcinoma [91].

As antibiotic resistance has become a global health problem, efforts are underway to identify and screen for new and effective antibiotics. A microarray for 132 gram-negative bacteria has been evaluated to detect genes for resistance to 75 clinically relevant antibiotics [92]. Frye et al. have developed a DNA microarray capable of detecting all antimicrobial resistance genes found at the National Center for Biotechnology [93]. Furthermore, a microarray has been use to identify *Helicobacter pylori* resistance to clarithromycin and levofloxacin, as well as to detect *CYP2C19* polymorphism [94]. It is reported that this microarray can be used for individual therapy detection as it has high specificity, reproducibility, and sensitivity [78]. In another study, an effort has been successfully undertaken to reduce antibiotic susceptibility testing assay time, as well as for rapid determination of minimum inhibitory concentrations of different antibiotics using a nanoliter-sized microchamber/microarray-based microfluidic (N-3 M) platform [95]. More recently, a commercially available microarray (IDENTIBAC AMR-ve) has been developed for determination of antibiotic-resistant clinical isolates of *Klebsiella pneumoniae,* and to identify genes associated with resistance to a wide range of antibiotics [96].

2.5 Identifying new drugs using microarray

Microarrays have been successfully used not only in various fields of medical research and for treatment, but also as useful platforms/tools for drug discovery. A general scheme for drug discovery and development is presented in **Figure 2**.

Small-molecule microarrays (SMMs) serve as a robust and novel technology that will have important applications in target-based drug discovery. In this technology, it is proposed that depending on the screening strategy, small molecules are either covalently or noncovalently immobilized onto a microchip. Hence, high precision robotic printers are used to automatically spot around 5000 molecules along a standard microscopic glass slide, with a spot diameter ranging between 80 and 200 μm. Therefore, a biomolecule of interest is tagged with a fluorophore, and then detected through a fluorescence-based readout. Using this SMM technology on a mammary tumor organoid model, multiple Malat1 ENE triplex-binding chemotypes have been identified, and selected compounds have been found to reduce expression levels of *MALAT1* [97]. This effort has demonstrated the plausibility of designing small molecules to investigate and treat *MALAT1*-driven type cancers.

An AbsorbArray is a small molecule microarray-based approach that allows for unmodified compounds to noncovalently adhere onto surfaces of an agarose-coated microarray to bind to RNA-motif libraries in a massively parallel format [98]. Using this platform, Hafeez et al. have designed a small molecule (TGP-377) that specifically and potently enhances vascular endothelial growth factor a (*VEGFA*) expression by targeting miR-377 and *VEGFA* mRNA [99].

Over the past decade, various drug screening platforms have been developed to control delivery of different drug candidates into target cells, including drug patterning, stamping, and microfluidic loading [100]. For example, a microarray-based screening system to test for effects of small molecules on mammalian cells utilizes an imaging-based readout. This system allows for conducting small-molecule screening for discovery of new chemical tools and of potential therapeutic agents [101]. In another example, a printed hydrogel is used in a high-throughput microarray-based
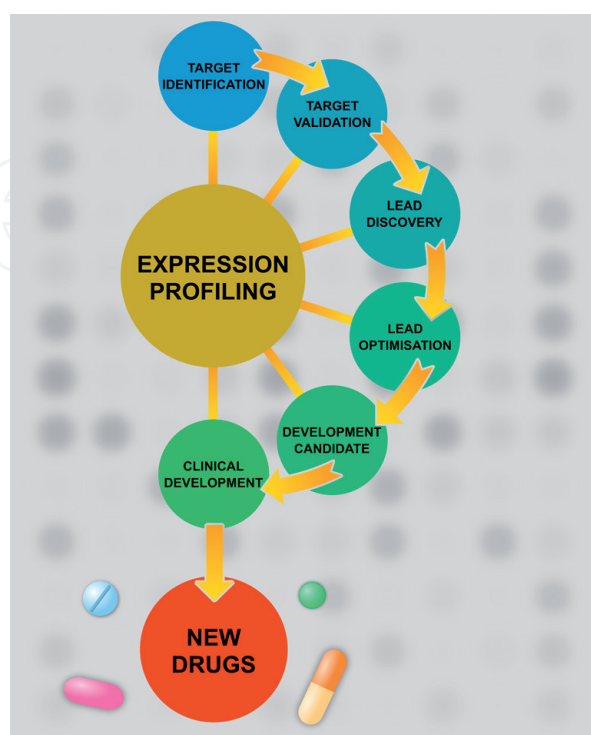
Figure 2.
Major steps undertaken in discovery and development of new drugs.

screening platform for rapidly and inexpensively identification of clinically promising lead compounds with inhibitory potentials [86]. Moreover, this platform can be used to quantify dose–response relationships of such inhibitors [102].

A schematic diagram of the drug discovery process using microarrays is presented in **Figure 2**.
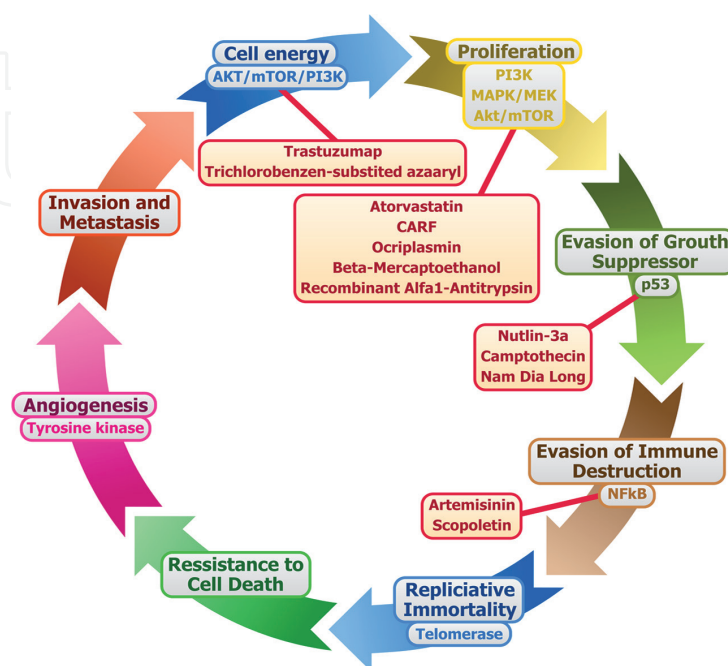
2.6 Microarrays and drug discovery for cancer

Microarrays are playing important roles in the discovery of critical drugs for the treatment of various forms of cancer. An overview of the scheme for anticancer drug discovery and development using microarrays is presented in **Figure 3**.

Microarray-based mRNA expression analysis has revealed that artemisinin induced iron-dependent cell death (ferroptosis) in an NCI cell line panel [103]. In this study, genes subjected to cluster analysis have been derived from different microarray hybridization platforms (Stanford, Affymetrix U95U95v2, U133, and U133A/U133) [87]. It is observed that *OGFOD1* and *TFRC* genes have exhibited comparable responses in Affymetrix microarrays U133 and U133A/U133B. In another microarray analysis study including 293 stomach tumor tissues and 196 normal tissues, it is found that two hub genes, Serpin Family E Member 1 (*SERPINE1*) and Secreted Protein Acidic and Cysteine Rich (*SPARC*), are significantly upregulated in gastric tissues, and are associated with poor outcomes [88]. Thus, this has demonstrated that transcriptome microarray datasets may facilitate early diagnosis of gastric cancer, and they may be used for pursuing effective treatment approaches [104].

Interestingly, scopoletin, a coumarin compound, is found to have an antiproliferative activity against tumor cells with ABC-transporter expression [89]. Furthermore, COMPARE and hierarchical cluster analysis of transcriptome-wide mRNA expression have supported the capacity of such compounds in drug development [105].

Furthermore, microarray analysis has provided evidence that the micro-RNA has-miR-542-5p can serve as a predictive biomarker, as well as a potential target for therapy in breast cancer [106]. Moreover, this microRNA acts via a mechanism involving the following target genes *YWHAB*, *LY9*, and *SFRP1* [90]. In another

Figure 3.
The hallmarks of cancer and drug discovery using microarrays.

study, it is reported that for patients with high-grade gliomas, microarray data from GSE4412 and GSE7696 datasets have identified differentially expressed prognostic genes between long-term and short-term survivors [91]. Thus, these genes have been deemed as potential biomarkers for prognostic, diagnostic, and therapeutic strategies [107]. Interestingly, atorvastatin treatment of HepG2 cells is reported to modulate 13 miRs identified in a microarray study [108].

Over the years, there have been several advances in design and analysis of microarray. For example, such advances have helped in the development of more specific biomarkers for prostate cancer in order to design effective therapeutic strategies [109]. It is found that urinary prostate cancer-derived exosomes could serve as promising sources of novel biomarker(s). In another study, fabrication of a microarray platform via a sandwich system has allowed for screening of 320 drug candidates as potential anti-cancer agents in *in vitro* experiments performed on MCF-7 breast cancer cells [110]. Furthermore, new bioinformatics tools have been used for microarray data analysis, and have led to the identification of *CDX2* as a prognostic marker for stages II and III colon cancer [111].

Interestingly, lncRNA-TTN-AS1, a novel vital regulator of esophageal squamous cell carcinoma, has been identified using microarray analysis, and found to correlate with overall survival [96]. This biomarker promotes *SNAIL1* and *FSCN1* expression binding to miR-133b, as well as interaction with mRNA, thereby leading to activation of a metastasis cascade [112]. Carstens et al. have developed a combinatorial chemotherapeutic drug-eluting microarray for tumor-initiating cancer stem cells capable of performing chemosensitivity screens using limited cell numbers [113]. In fact, a lncRNA microarray analysis using hepatocarcinoma HCC cells has demonstrated that HNF1A-AS1 is a direct transactivation target of *HNF1α,* and it may have beneficial effects in the treatment of this form of cancer [114]. A pathway analysis of microarray data has identified a transient receptor potential vanilloid (*TRPV*) 2 as a novel therapeutic target for esophageal squamous cell carcinoma. *TRPV2* depletion is found to down-regulate WNT/β-catenin signaling-related genes, as well as basal cell carcinoma signaling-related genes [115]. In another development, using small molecule microarrays, protein–protein interaction inhibitors of *BRCA1* that can be directly administered to tumor cells have been identified [100]. In fact, these compounds have proven to be useful in cancer therapy by targeting BRCA1/PARP-related pathways involved in DNA damage and repair response [116].

In other cancer drug discovery studies, analysis of microarray data has revealed that manzamine (or Manz A) is found to have an antiproliferative effect on human colorectal carcinoma cells, wherein it reduces expression of genes involved in cell survival, induces apoptotic cell death, and inactivates epithelial to mesenchymal transition (EMT) [117]. Furthermore, Manz A is proposed as a potential anticancer drug for colorectal cancer patients by blocking tumors undergoing EMT process and developing distal metastasis. In another effort, the Collaborator of ARF (CARF) protein has been discovered by microarray analysis as a new target of miR-451, and that it mediates its tumor suppressor function both in normal and stressed biological states [118].

In a comparative study, RNA-seq and qPCR-based arrays were found to be better suited than transcriptomic cDNA microarrays in assessing G protein-coupled receptor (*GPCR*) expression with implications for *GPCR* biology and drug discovery [119]. A gene expression omnibus (GEO) database for mRNA microarray data was used for discovery of potential biomarkers in HER-2 positive breast cancer patients who received a neoadjuvant trastuzumab treatment [120]. Furthermore, a combination therapy of trastuzumab and anti-Wnt or hormone therapy could serve as an effective treatment for breast cancer. In addition, expression microarray analysis led to the identification of internalizing antibodies (CD73 mAbs) for basal breast

cancer cells [121]. Thus, these mAbs were found to bind to basal-like breast cancer cell surface receptors of high affinity and specificity, as well as promoted receptor-mediated endocytosis with potential applications in basal-like breast cancer treatment [106]. Following microarray gene expression profile analysis, ocriplasmin, β-mercaptoethanol, and recombinant α 1-antitrypsin were identified as potential drugs for the treatment of papillary thyroid cancer [122]. Moreover, microarray profiling assisted in identifying the cytotoxicity mode of action involved in apoptosis of MCF-7 cells following treatment with Nam Dia Long (NDL), a Vietnamese traditional formula [123].

In other studies, genomics and proteomics data have revealed that the ribonucleotide reductase regulatory subunit M2 (*RRM2*) is a novel target of sorafenib in hepatocellular carcinoma [124]. Whereas, a cDNA microarray analysis has identified trichlorobenzene-substituted azaaryl compounds as novel *FGFR* inhibitors with capabilities in downregulating genes associated with cell cycle progression, and in upregulating genes associated with autophagy pathway in bladder cancer [125].

2.7 Microarrays and drug discovery for various other pathologies

Microarrays have been widely used for screening, identifying, and discovery of drugs for various pathologies. A summary of various microarrays used in the discovery of relevant drugs for some of these pathologies will be presented.

A pharmacogenomics corticosteroid model in rat liver was quantified using microarrays and mass spectrometry-based proteomics [126]. Furthermore, corticosteroid-regulated gene expression was observed at mRNA and protein levels, and acting via mechanisms influencing key turnover processes. In another study, an Affymetrix DMET Plus GeneChip microarray platform was found to be useful in discovery of new genetic variants involved in risperidone-induced hyperprolactinemia based on correlations of genetic variations with target genes of interest [127]. In yet another innovative approach, baseline blood sample microarray data and machine learning were exploited to develop a predictive model for lithium treatment response in bipolar patients based on pre-treatment gender and gene expression data [128]. In fact, this predictive model can be extended not only for other therapeutic drug classes, but also for discovery of new biomarkers [113].

Using an Affymetrix_Hugene_1.0_ST microarray, latrophilin (LPHN) receptors have been identified as novel bronchodilator targets for asthma [114]. Moreover, a single nucleotide polymorphism (SNP) in *LPHN1* correlated with asthma along with higher *LPHN1* expression in lung tissue [129]. Just as important, microarrays, based on normalized cDNA libraries, have been used to successfully discover novel genes as potential candidates for drug targeting. In one study whereby a mouse model of immunoglobulin A nephropathy was used, the single most important drug targets in nephritis, namely up-regulated G-protein coupled receptors (*GPCR*s), have been identified [130]. In other efforts, novel biomarkers related to ageing and age-related diseases have been also discovered using microarrays. For example, Lamb et al. generated a large public database of signatures of drugs and of genes by identifying small molecules with potential applications for the treatment of Alzheimer's disease [131]. Likewise, a microarray study was conducted to compare gene expression of major metabolic tissues in mice, rats, and obese cynomolgus monkeys, and it was observed that a modified growth differentiation factor 15 (GDF15)-Fc fusion proteins could serve as potential therapeutic agents for obesity, and for treatment of related comorbidities [132]. Moreover, chemical microarray-assisted high-throughput screening of potential drugs has contributed for rapid identification of four peptoids as fibroblast growth factor receptors (FGFR) agonists with potential applications in clinical use [113].

In a new twist, a phenotypic microarray (PM) technology has been used to measure *Candida albicans* metabolic activity in the presence/absence of acetylcholine, thus paving the way for discovery and screening of compound libraries for novel anti-fungal drugs [133]. While glycan microarrays were found useful in supporting analysis of receptor-binding specificity for glycan-binding pathogens to tackle viral infections, as well as for appropriate design of viral vectors for therapeutic applications [134, 135]. Along the lines of combining different technologies, microarrays were integrated with high-throughput proteomics to promote discovery of transthyretin as a potentially valuable target for rhabdomyolysis-induced acute kidney injury, as transthyretin induced apoptosis by decreasing accumulation of reactive oxygen species (ROS) [136]. In another study, microarray analysis revealed that the nitric oxide–sensitive soluble guanylyl cyclase improved both diastolic cardiac function and hemodynamics, as well as decreased susceptibility to ventricular arrhythmias in animal models [137]. Whereas, Takahiro et al. reported on a novel method to analyze glycan profiles of hemagglutinin using a lectin microarray that served as a highly sensitive and simple tool for glycan profiling of viral glycoproteins [138]. Similarly, using a high-density peptide microarray, designed using linear peptides and consequentially conformational epitopes, specific diagnostic peptides for the Zika virus were identified, and this approach could be rapidly adapted to other pathogens [139]. In another microarray study along with use of the WGCNA (weighted gene co-expression network analysis) method, genes related to inflammatory and immune responses with critical roles in rheumatoid arthritis pathogenesis have been identified, and both sanguinarine and papaverine were deemed as having potential therapeutic effects on rheumatoid arthritis [140].

In another innovative approach, a meta-analysis of polymyositis and dermatomyositis microarray data has revealed that four novel genes and ten SNP-variant regions could be used either as candidates for potential drug targets or as biomarkers [141]. Interestingly, microarray analyses have indicated that SAM-competitive EZH2 inhibitors in cancer cells induced genes related to cholesterol homeostasis in hepacellular carcinoma [142]. Moreover, gene expression microarray studies have that revealed that T2DM-connected genes as alternative drug targets. Furthermore, interatomic and toxicogenomic have helped to identify signaling pathways involved in disease pathophysiology [143]. An integrative gene expression microarray meta-analysis has provided valuable information about novel potential host factors that can modulate chronic HBV infection, and may serve as potential targets for the development of novel therapeutics such as the activin receptor-like kinase inhibitor [144].

In other innovative platforms, non-natural amino acid peptide microarrays were developed for discovery of Ebola virus glycoprotein affinity ligands, and this system could be used for rapid development of peptide-based antivirals for other diseases [145]. On the other hand, Kusi-Appiah et al. developed a method in order to generate quantitative dose–response curves from microarrays of liposomal small molecules [129]. This method was found to control dosages of small lipophilic molecules provided to cells by varying sub-cellular volumes of surface-supported lipid micro- and nano-structure arrays manufactured using nanointaglio printing [146].

In other studies, microarrays have been used to select either cooperative or non-cooperative peptide pairs for modulating enzyme functions for use in both drug discovery and biocatalysis [147]. Specifically, new peptides promoting inhibition of the target enzyme are selected by jointly using them along with a primary inhibitory peptide. Furthermore, a quantitative PCR-based microarray has been used to assess differences in expression levels of miRNA from plasma of women with or without endometriosis, and a potential diagnostic marker, hsa-miRNA-154-5p, for this disease is identified [148]. In another study, altered gene expression profiles in peripheral blood mononuclear cells (PBMCs) of type 1 diabetes (T1D)

are identified using integrated analysis of different microarray studies, thereby offering a new strategy for either preserving or improving β-cell function [149]. Moreover, microarray analysis has allowed for the identification of an aurora kinase A (AURKA) gene involved in cell cycle regulation that could serve as a potential biomarker for predicting poor prognosis in liposarcoma [150].

Microarrays have been used to identify drugs for various other diseases. For example, collagenase is demonstrated to play an important role in ischemia stroke through TNF and IL1B, and a DNA microarray has identified anakinra and nitric oxide as small molecule drugs that are closely associated with this disease [151]. While protein microarrays have been used as platforms to "target hop", critical for identifying small molecules that bind to, and compete with, domain–motif interactions [152]. In fact, Bae et al. have used this platform to identify a novel compound, EML405, via its interaction with the Tudor domain-containing protein Spindlin1, SPIN1. Furthermore, microarray screening has identified a retinoid derivative Tp8 that promotes anti-hepatitis C virus activity via restoration of the gastrointestinal glutathione peroxidase (GI-GPx) [153]. In a different study, a small–molecule microarray (SMM)–based screening has contributed to the identification of an inhibitor (a degradation product from a commercial screening collection) of the "undruggable" small ubiquitin–like modifier (SUMO) E2 enzyme Ubc9 [154]. This latter discovery provides a viable example of the significant pharmacological importance of this SMM screening strategy.

There are additional examples of the impact of microarray analyses in identifying valuable drugs against serious human diseases. GSE7621 microarray data from the GEO database have allowed for the identification of 49 novel small molecular drugs that can target several sub-pathways of Parkinson's disease [155]. Moreover, this strategy has allowed for predicting potential therapeutic properties of novel agents, such as ketoconazole and astemizole, in Parkinson's disease via targeting of key enzymes in the arachidonic acid metabolism [138]. In another microarray study, cyclosporine, ethinyl, and tretinoin have been identified, using the Linear Models for Microarray package, as potential targets for treating pulmonary thromboembolism [156]. Whereas, the effect of astragalosides (AST) in rheumatoid arthritis has been elucidated following microarray analysis of critical differentially expressed lncRNAs involved in this disease, wherein four lncRNAs have been selected as critical therapeutic targets for AST [157]. In a recent study, microarray analysis has revealed that the synthetic lipid AM251 inhibits SMAD2/3 and p38 mitogen-activated protein kinase (MAPK), as well as suppresses EMT of renal tubular epithelial cells [158]. Whereas emodin, a Chinese herb-derived compound, is found to suppress excessive responses of macrophages, and it is capable of restoring macrophage homeostasis in different pathologies [159]. Moreover, findings of a microarray analysis have revealed that medroxyprogesterone acetate (MPA), a progestin-based hormonal contraceptive designed to mimic progesterone, increases expression of genes related to inflammation and cholesterol synthesis, as well as those genes associated with both innate immunity and HIV-1 susceptibility [160]. Finally, integrative microarray data have been exploited to identify eight hub genes and one potential nanomedicinal drug, Selenocysteine, that promotes cartilage regeneration [161].

3. Next generation sequencing for drug discovery

Next generation sequencing (NGS) is the term used for massive parallel sequencing experiments that can be conducted using DNA, RNA, or miRNA. NGS has revolutionized clinical and research studies by enabling sequencing of whole human genomes within a single day.

This powerful NGS can be used in several different areas. For example, NGS can be used in clinical settings for identifying genetic variants with high specificity and sensibility, thus allowing for detection of mosaic mutations that could not be previously identified by Sanger sequencing [162]. In the field of microbiology, NGS can be used for identifying and characterizing pathogens, including novel strains or mutants, thereby allowing for linking a pathogen or a new pathogenic strain to an outbreak in a specific region or to a particular individual(s) [163]. The role of NGS in the field of oncology is quite significant, as this technology can be used for pursuing personalized medicine, in particular for developing targeted therapies for specific cancers correlated with individual genetic profiles of patients. Moreover, NGS is highly useful for diagnosis, and for classification of different types of cancer in both adults and children [162, 164, 165].

Furthermore, NGS is highly versatile, primarily for the diversity of analysis that can be undertaken, as well as to numbers and types of biological samples that can be analyzed. A listing of major types of analyses that can be undertaken, as well as of types of biological samples used in NGS are presented in **Table 2**.

As for drug discovery, NGS has been successfully used in various areas of drug discovery, beginning with target identification, compound screening,

Analysis type	Purpose(s)	Biological sample(s)
Targeted gene sequencing	Identify genetic alteration(s) for a specific set of gene region(s) or SNP(s)	Cell cultures; whole blood; serum; plasma; fresh/frozen tissue; formalin-fixed paraffin-embedded tissue
Whole exome sequencing	Evaluate variation(s) present in coding region(s) of DNA (exomes)	Cell cultures; whole blood; fresh/frozen tissue; formalin-fixed paraffin-embedded tissue
Whole genome sequencing	Identify variations present in the whole genome of an organism(s)	Cell cultures; whole blood; fresh/frozen tissue; formalin-fixed paraffin-embedded tissue
miRNAseq	Identify miRNAs and their expression level(s)	Cell cultures; whole blood; serum; plasma; fresh/frozen tissue
RNAseq	Determine expression levels of whole genes present in an organism	Cell cultures, Whole blood, serum, plasma, fresh/frozen tissue
CHIPseq	Chromatin immunoprecipitation sequencing allows for identifying alterations at DNA –binding sites of different transcriptional factor(s) or protein(s)	Cell cultures; fresh/frozen tissue
Copy number alterations/ variations (CNVs)	Detect duplication(s), deletion(s), translocation(s), or inversion(s) of one or more genes	Cell cultures; whole blood; fresh/frozen tissue; formalin-fixed paraffin-embedded tissue
Methylation sequencing	Evaluate whole methylation pattern(s) in CpG, CHG, and CHH regions across a genome	Cell cultures; whole blood; fresh/frozen tissue; formalin-fixed paraffin-embedded tissue

Table 2.
Types of NGS analysis, purpose(s), and biological samples used.

biomarker discovery, identification of biopharmaceuticals, drug resistance, and vaccine discovery [166–168]. Those steps involved in drug discovery where NGS could be of particular use are presented in **Figure 4**.

3.1 Target identification

In recent years, NGS has been valuable in the identification of different genetic alterations of a pathogen/pathology that can be useful for targeted treatment. The versatility of NGS allows for evaluating genomic regions using genomic analysis, transcriptomics, RNAseq, and miRNA seq in order to identify gene(s) and their regulation(s)/functionality(ies) in response to different disease conditions, which in turn could be used for target identification [169].

Analysis of genetic variant(s) is yet another important approach for identifying mutations in rare diseases, as these could then be used for treatment of such target(s) [170, 171]. Epigenetic studies, such as methylation analysis or CHIP-seq analysis, known to be altered in different pathologies, could also aid in identifying targets for specialized treatments/therapies [172, 173].

NGS has been widely used for gene to target identification for treatment of cancer. As it is well known, the National Comprehensive Cancer Network (NCCN) has several guidelines for NGS target identification used for treatment of various types of cancer. These include targeting genes for lung cancer (*EGFR, ALK, ROS1, BRAF*, and *PDL1*) [174], colorectal cancer (*NRAS/HRAS/KRAS, BRAF*, *HER2*, *MLH1, MSH2, MSH6,* and *PMS2*) [175], breast and ovarian cancers (*BRCA1/2, TP53, STK11, PTEN, CDH1, PALB2*, among others) [176]. By identifying mutations in each of these genes, clinicians are able to treat patients with specific targeted treatments. In Waldenström's macroglobulinemia, NGS has been employed in evaluating genomic variations that could better inform treatment of patients, and that would ultimately lead to better outcomes. It is observed that patients with recurrent somatic mutations in genes of myeloid differentiation factor 88 (*MYD88*) and chemokine receptor type 4 (*CXCR4*) demonstrate different responses to the same treatment, and thus these genes serve as clinical determinants of clinical
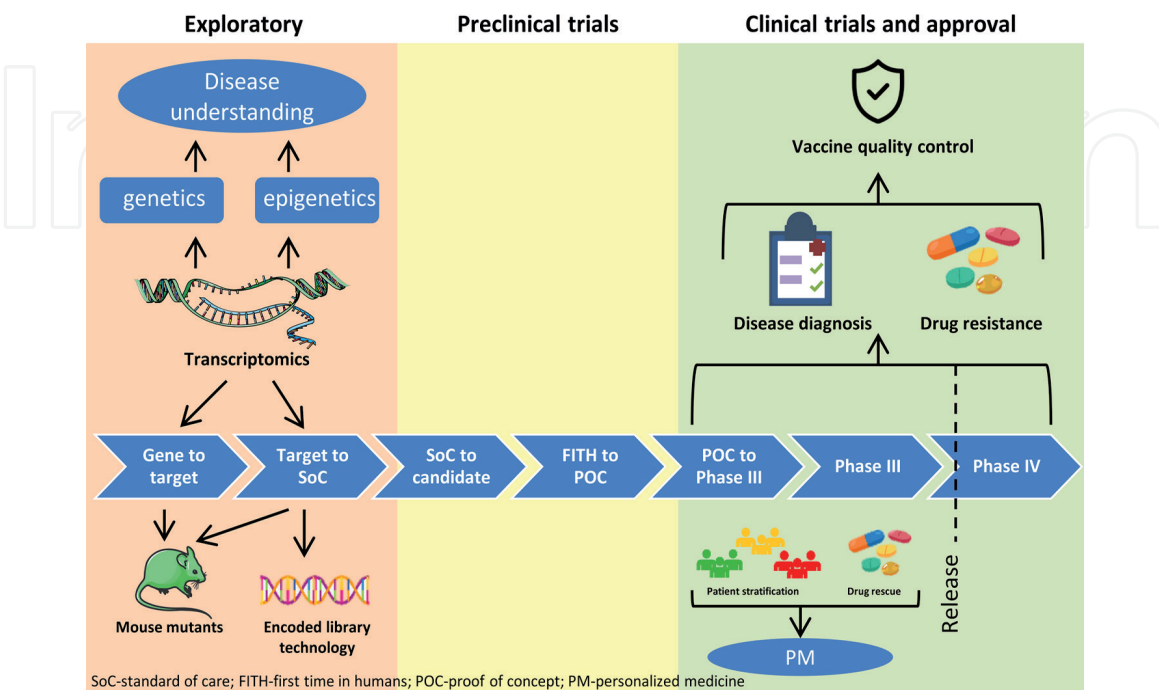
Figure 4.
Various steps involved in drug discovery whereby NGS can be of particular benefit.

presentation [154]. Therefore, a treatment algorithm can be used, based on the mutational status of a patient, in a clinic to adjust targeted treatment [177].

Although cancer has been the most widely studied disease over the last decade, other diseases have employed this approach to identify improved therapies/treatments for each individual patient. For example, Tshibangu-Kabamba et al. have used NGS for evaluating antimicrobial resistance (AMR) of different strains of *Helicobacter pylori*, as well as for determining antimicrobial susceptibilities of these bacterial strains [155]. Whole genome sequencing has aided in identifying several variants in AMR genes, such as *pbp1A* (T558S, F366L), *gyrA* (A92T, A129T), *gyrB* (R579C), and *rdxA* (R131_K166del). This has been instrumental in determining susceptibility of these strains to specific drugs [178].

RNA-seq technology has been used for profiling of host, bacteria, and SARS-CoV-2 virus outbreaks in New York City [156]. It is reported that RNA-seq results are similar to those of RT-PCR. In addition, it is observed that SARS-CoV-2 samples seem to carry other types of viruses. Interestingly, it is also observed that there are gene expression dysregulation in viral response pathways, innate immune responses, and interferon signaling that could explain different responses of patients to the same antiviral drugs [179].

In another study, NGS has been used to identify a targeted treatment for a patient suffering from an immune dysregulation syndrome. As a result, a new germline mutation in the *CTLA4* (Cytotoxic T-Lymphocyte Associated Protein 4) gene, susceptible to the drug abatacept, has been identified [180].

3.2 Target to standard of care

In this step of drug discovery, NGS plays an important role, mainly due to its ability to assess multiple gene alterations within a short period of time. Moreover, the Food and Drug Administration (FDA) has approved NGS testing in clinics. One such example is the case of using a hybrid capture NGS assay for evaluating non-small cell lung cancer in patients. Using this assay, Schatz et al. have diagnosed 417 patients based on both genetic alterations and tumor burden. This approach has made it possible to use specific treatments based on tumor burden values if no actionable genetic alteration is detected [181, 182]. Furthermore, Klowak et al. have used NGS in a pilot study to identify pathogens in neonates suspected of having sepsis. They have proposed an NGS-based protocol for implementation in clinics to accurately and rapidly identify those pathogens affecting neonates, as well as to provide better treatments [183]. Yet in another example, an NGS panel, consisting of seven fusion genes and seven genes with frequent copy number changes, has been used to diagnose 113 sarcoma patients with 97% sensitivity and 100% specificity. This has rendered this gene panel as a highly promising toll for implementing gene targets in standard of care for sarcoma patients [184]. There are several other studies demonstrating the utility of NGS testing in identifying targets that could be actionable by either specific drugs or that could be implemented as specific targets for standard of care for particular diseases [185–189].

3.3 Compound screening

In recent years, a common method used for compound screening during drug discovery is "encoded library technology" (ELT) [190]. ELT is based on DNA binding to members of a small molecule library of chemical compounds. This DNA tag, serving as an amplifiable identification barcode, is unique to each compound/organic ligand, thus rendering it possible for its incubation with specific protein targets of interest. Subsequently, these organic compounds/ligands are washed away

based on their affinities to the target; thus, compounds/ligands with high affinities for the target are enriched, and identified by NGS sequencing of PCR products [190]. This approach allows for both constructing and screening of combinatorial libraries of large volumes, thus facilitating rapid discovery of ligands to various different protein targets. ELT is used in several clinical areas, mainly for cancer, but also for various diseases, as it is a rapid and economical screening system of organic compounds [191–194].

Recently, Lemke et al. used ELT and virtual computation library screening methods, DNA-encoded chemical libraries (DECL), to identify inhibitors for poly-ADR-ribose polymerase member 10 (*PARP10*). In effect, they integrated DECL screening with structure-based computational methods to streamline the development of leading compounds. Thus, following DECL screening, they observed that a compound with an A82-CONHMe-B54 motif yielded the best result. Therefore, they screened over 10,500 virtual compounds, and selected ten compounds for synthesis. These compounds were assessed for *PARP10* inhibition, and they found two compounds with promising results [195]. In another study, Reidenbach et al. attempted to identify compounds against Prion disease, a neurodegenerative disease with no therapeutic options; however, the only benzimidazole compounds identified demonstrated low affinities [196]. Whereas, Cuozzo et al. screened a DECL library of 225 million compounds, and identified a single compound (X-165) with a high activity against the production of lysophosphatidic acid, and this compound has been approved by the FDA for Phase I Clinical trials [197]. In other examples of using this strategy, Dawadi et al. discovered a thrombin inhibitor using DECL [198], while, Kung et al. identified two compounds that presented inhibition/binders to e Nα-terminal acetyltransferase (Naa50) using ECL library screening [199].

3.4 Undruggable targets and NGS

As mentioned above, an "Undruggable" target is a term given to sets of proteins that cannot be targeted by a specific treatment, yet they can be exploited for the development of treatments for various diseases.

Among these undruggable targets are non-enzymatic proteins, transcriptional factors, regulatory proteins, and scaffolding proteins [200, 201]. One such undruggable target is the Kristen Rat Sarcoma (KRAS) protein encoding a viral oncogene, detected in non-small cell lung cancer (NSCLC). Recently, KRAS mutations have been successfully targeted using different approaches, such as inhibition of downstream effectors, epigenomic approaches, post-translational modifications, and high-affinity KRAS binders, among others, wherein direct pharmacological inhibition of a *KRAS p.G12C* mutation is deemed possible, thus serving as an effective targeted treatment available for patients with advanced NSCLC [202]. Moreover, other members of the RAS family are deemed as undruggable targets in cancer, and several approaches have been used. Kato et al. have used NGS to evaluate the mutational status of 1937 patients with different cancers, and have observed that over 20% presented *RAS* alterations. Unfortunately, poor overall survival has been observed in spite of various treatment options that are offered; however, a better survival is observed for patients treated using a combined therapy targeting MAPK and non-MAPK pathways [203]. Among other undruggable targets, MYC and TP53 are known to have no enzymatic activities, and are located intracellularly. However, a Phase III trial is undergoing for TP53 using the APR-246 drug for myelodysplastic syndrome, and although there are no current clinical trials for MYC, an anti-MYC compound, OmoMYC, has been validated in multiple preclinical studies [204].

In other efforts, Zhou et al. have proposed the use of neoantigens, collected from patients with gastric cancer, for targeted therapies for gastric cancer disease [181]. In this study, six highly mutated genes along with high frequency HLA alleles have been identified, thus rendering neoantigens of these six genes as possible targets for immunotherapy of gastric cancer [205]. In another study on neuroblastoma, it is reported that a *MYCN* gene can be transformed into a drug-gable target by targeting different regulators of its pathway, such as β-estradiol and MAPK/ERK [206].

3.5 Drug resistance and NGS

Using NGS, a new gene was identified in *Acinetobacter baumannii* strain 863 conferring multi-drug resistance to this bacterial pathogen [207]. In another study, an antibiotic resistance signature of 25 genes was differentially expressed in *Staphylococcus aureus* [208]. Furthermore, it was reported that NGS might be successfully used for early identification of mutations related to drug resistance in transplant patients treated for cytomegalovirus [209].

In other studies, metagenomics NGS assays have been used to identify microbial composition and antibiotic resistance in water samples of Puget Sound (Washington State), and have reported that this could serve as a reliable protocol for providing accurate information on bacterial composition and antibiotic resistance in water samples [210]. Leprohon et al. have reviewed all critical information relevant to drug resistance and to resistance mechanism(s) in Leishmania infections generated from NGS analysis [211]. Furthermore, NGS has been successfully used for testing for HIV-1 drug resistance, although such studies are yet to be standardized [212–216]. Likewise, NGS and pyrosequencing have been used to investigate resistance of the *Influenta A* virus to baloxavir [217]. Moreover, NGS has also been used for detection of those *H. pylori* clones that are resistant to levofloxacin [218]. While RNA-seq data have been mined to identify novel fusion genes in gastrointestinal stromal tumor patients with resistance to imatinib [219].

4. DrugBank

Another important set of tools in drug discovery are the collective databases of drugs with detailed information about drugs, including their actions and targets. One such database is DrugBank, launched back in 2006, as it combines various resources offering clinical information, including chemical information about drugs and resources [220]. The main focus of DrugBank is to offer information relavent to mechanistic data, structures, and sequences about drugs and their targets. Furthermore, this resource is capable of providing tools for viewing, sorting, and searching both sequence and structure data [220]. Lately, DrugBank database has been further improved, as it now can offer information about 1467 FDA-approved drugs, 123 biotech drugs, 69 nutraceuticals, 4774 small molecule drugs, and 3116 experimental or unapproved drugs. There is also information related to withdrawn [57] and illicit [188] drugs. Furthermore, it has a higher drug target database for FDA-approved drugs, which includes 1565 non-redundant protein/DNA targets [221].

Due to its wealth of information, DrugBank has been used for a variety of drug applications, including target prediction [222], *in silico* discovery [223], metabolism prediction [224], docking or screening [225], as well as new uses of old drugs [226]. Additional applications are presented in **Table 3**.

Type of application	Drug tested	Disease	Reference(s)
Drug-target identification	Traditional Chinese medicine derived from *Trachelospermum jasminoides*	Rheumatoid arthritis	[227]
Molecular docking and simulation studies	Mitoxantrone, Leucovorin, Birinapant, and Dynasore	SARS-CoV-2 M ^{pro}	[228]
Virtual drug screening	Repositioning Dequalinium	hM$_2$ allosteric modulation	[229]
Drug metabolism prediction	Drugs related to P450 cytochrome enzymes	Seniors' Metabolism of Medications and Avoiding Adverse Drug Events	[230]
Drug screening/ discovery	All drugs correlated to viral proteins	SARS-CoV-2	[231]
Molecular docking, drug resistance	Carbapenems	*Acinetobacter baumannii* OXA class enzymes	[232]
Molecular docking, molecular dynamic simulation	Approve drug libraries for ACE2	SARS-CoV-2	[233]
In silico screening	Glycoprotein inhibitors	SARS-CoV-2	[234]
Drug repositioning	Drugs that target genes in pathways for treating depression	Treatment of resistant depression	[235]
Pharmacological analysis	JianPi Fu Recipe	Colon cancer LoVo cells metastasis	[236]
Multi regulatory pathways construction	Pivotal Drugs for pancreatic cancer	Pancreatic cancer	[237]
Drug repurposing	Drugs that inhibit proteases	SARS-CoV-2	[238, 239]
Drug repurposing		Hypertension	[240]
Pharmacological drug mechanism	Aloperine	Cardiovascular disease	[241]
Drug screening	Drugs targeting immune-related genes	Cervical cancer	[242]

Table 3.
Applications of the DrugBank database.

Although **Table 3** presents only a few studies employing the DrugBank database, a PubMed search for DrugBank has identified at least 505 published articles, from 2006 until 2020.

5. NGS in SARS-CoV-2 drug discovery

As infections with the SARS-CoV-2 virus have become more aggressive, there is an urgent need for evaluating different drugs that may contribute to a better and effective treatment of this infection. The majority of drugs used for SARS-CoV-2 treatment are drugs currently in use for treatment of other diseases [243–245], and these have been evaluated for their efficacy using computational drug discovery analysis [246–248].

Although NGS has been used primarily for genome identification of SARS-CoV-2 [249–252], as well as for evaluation of mutations developed during viral spread in different countries [253–255], there are some studies wherein RNA sequencing is used for identifying new drug treatments. One such study has used NGS for evaluation of affected genes during SARS-CoV-2 infections. In this study, different genes involved in RNA regulation, histone remodeling, cellular signaling, and chromatin remodeling are identified. Some of these identified genes have demonstrated either pro- or antiviral activities; thus, these genes could serve as potential tools for different therapies or vaccines [256]. In another study, a shotgun metatranscriptomics RNA sequencing technique is used for a cohort of New York SARS-CoV-2 infected patients, and have identified host-responses to SARS-CoV-2 infections in different pathways such as interferon, ACE, olfactory, and hematological pathways [179]. Moreover, they have also analyzed risks associated with angiotensin blockers and ACE inhibitor treatments in SARS-CoV-2 infected patients [179].

6. Features of microarrays and NGS and their relevance in drug discovery

In general, there are a variety of features for each of microarrays and NGS that render these platforms highly valuable in the arena of drug discovery and therapeutics, and these are summarized in **Table 4**.

Microarrays offer various advantages including expression analysis of cells or tissues at different states of disease, pharmacogenomics, toxicogenomics, and as well as for analysis and identification of SNPs. The microarray technology is useful for obtaining a good amount of information from small volume samples, and it is quite valuable for use in incorporating low-cost high-throughput assays in the drug discovery process. However, this technology has a number of disadvantages. These include high costs and long timelines, particularly related to re-design of microarray chips to include newly discovered genetic targets.

In comparison to microarrays, NGS offers more flexibility and higher cost-efficacy. In particular, this technology allows for identification of targets, screening of large numbers of compounds for use in therapeutics or treatments, as well as for identifying of unique biomarkers useful for discovery of new drug targets.

	Microarrays	**NGS**
Advantages	Expression of thousands of genes simultaneously; Low sample consumption; Easy sample preparation and control of experimental conditions; Data variability	High sensitivity; Quantitative; High dynamic range; No hybridization
Disadvantages	Competence required for data normalization and analysis; Limited dynamic range; Low sensitivity; Competitive hybridization	Complex sample preparation; Complex technology infrastructure required; High cost
Applications	Biomarker identification; Gene discovery; Vaccine development	Target identification; Compound screening; Biomarker identification; Drug resistance; Vaccine discovery

Table 4.
Benchmarks for NGS and microarrays in drug discovery.

7. Conclusions

Overall, although the word "limitation" still floats around, and with only 5% of novel molecular compounds are ultimately selected to enter the drug and therapeutic marketplace, new innovations in science and technology are critical in the arena of drug discovery and therapeutics. It is these ongoing research advances and technological innovations that will empower scientists to continue on in the pursuit of additional and more sophisticated, reliable, and efficient molecular tools, such as NGS and microarrays, that will be useful in the arena of drug discovery and therapeutics. These efforts, innovations, and technologies will undoubtedly continue to revolutionize the drug discovery industry that will aid in identifying better and more effective drugs, at much lower costs, and within shorter periods of time.

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Conflict of interest

The authors declare no conflict of interest.

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