Chapter

Microarrays and NGS for Drug Discovery

Laura-Ancuta Pop, Oana Zanoaga, Paul Chiroi, Andreea Nutu, Schuyler S. Korban, Cristina Stefan, Alexandru Irimie and Ioana Berindan-Neagoe

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

**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 alecstinib 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
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
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 SNAI1 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 α1-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 (GPCRs), 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 hepatocellular carcinoma [142]. Moreover, gene expression microarray studies have 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)
Microarrays and NGS for Drug Discovery
DOI: http://dx.doi.org/10.5772/intechopen.96657

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 Ubε9 [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](image_url)

**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 Na-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 relevant 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.
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 anti-viral 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; | High sensitivity; |
| Low sample consumption; | Quantitative; |
| Easy sample preparation and control of experimental conditions; | High dynamic range; |
| Data variability | No hybridization |
| **Disadvantages** | Complex sample preparation; |
| Competence required for data normalization and analysis; | Complex technology infrastructure required; |
| Limited dynamic range; | High cost |
| Low sensitivity; | |
| Competitive hybridization | |
| **Applications** | Target identification; Compound screening; |
| Biomarker identification; | Biomarker identification; Drug resistance; |
| Gene discovery; | Vaccine development |
| Vaccine development | 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.

Funding

The funding for this book chapter is ensured by the PCCDI National Project: Genomic mapping of the population in the areas radioactively contaminated with heavy metals in order to increase national security- ARTEMIS.

Conflict of interest

The authors declare no conflict of interest.
Author details

Laura-Ancuta Pop¹, Oana Zanoaga¹, Paul Chiroi¹, Andreea Nutu¹, Schuyler S. Korban², Cristina Stefan³⁴, Alexandru Irimie⁵ and Ioana Berindan-Neagoe*¹

¹ Research Center for Functional Genomics, Biomedicine and Translational Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

² Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

³ SingHealth Duke-NUS Global Health Institute (SDGHI), Duke-NUS Medical School, Singapore

⁴ African Medical Research and Innovation Institute, Cape Town, South Africa

⁵ Department of Surgical Oncology and Gynecological Oncology, University of Medicine and Pharmacy Iuliu Hatieganu, Cluj-Napoca, Romania

*Address all correspondence to: ioananeagoe29@gmail.com

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
References

[1] Doroshow JH, Kummar S. Translational research in oncology--10 years of progress and future prospects. Replace with: Nat. Rev. Clin. Oncol. 2014;11(11):649-62.

[2] Kunnumakkara AB, Bordoloi D, Sailo BL, Roy NK, Thakur KK, Banik K, et al. Cancer drug development: The missing links. Exp. Biol. Med. 2019;244(8):663-89.

[3] Wilding JL, Bodmer WF. Cancer cell lines for drug discovery and development. Cancer Res. 2014;74(9):2377-84.

[4] Roy PS, Saikia BJ. Cancer and cure: A critical analysis. Indian J. Cancer. 2016;53(3):441-2.

[5] Bordoloi D, Sailo, B.L., Manteghi, N., Padmavathi, G., Kunnumakkara, A.B. Introduction and basic concepts of cancer. Cancer Cell Chemoresist. Chemosensit. 2018.

[6] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: Cancer J. Clin. 2018; 68(6):394-424.

[7] Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int. J. Cancer. 2019; 144(8):1941-53.

[8] Vasan N, Baselga J, Hyman DM. A view on drug resistance in cancer. Nature. 2019;575(7782):299-309.

[9] Eder J, Herrling PL. Trends in Modern Drug Discovery. Handbook of Exp. Pharmacol. 2016;232:3-22.

[10] Li JW, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier? Science. 2009;325(5937):161-5.

[11] Chan HCS, Shan H, Dahoun T, Vogel H, Yuan S. Advancing Drug Discovery via Artificial Intelligence. Trends Pharmacol. Sci. 2019;40(8):592-604.

[12] Overington JP, Al-Lazikani B, Hopkins AL. How many drug targets are there? Nat. Rev. Drug Discov. 2006;5(12):993-6.

[13] Adams CP, Brantner VV. Estimating the cost of new drug development: is it really 802 million dollars? Health affairs. 2006;25(2):420-8.

[14] Mariotto AB, Enewold L, Zhao J, Zeruto CA, Yabroff KR. Medical Care Costs Associated with Cancer Survivorship in the United States. Cancer Epidemiol Biomarkers Prev. 2020 Jul;29(7):1304-1312.

[15] Prasad S, Gupta SC, Aggarwal BB. Serendipity in Cancer Drug Discovery: Rational or Coincidence? Trends Pharmacol. Sci.. 2016;37(6):435-50.

[16] Workman P, Antolin AA, Al-Lazikani B. Transforming cancer drug discovery with Big Data and AI. Expert Opin Drug Discov. 2019;14(11):1089-95.

[17] Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. New England J. Med. 2015;372(21):2018-28.

[18] Gyawali B, Hwang TJ, Vokinger KN, Booth CM, Amir E, Tibau A. Patient-Centered Cancer Drug Development: Clinical Trials, Regulatory Approval, and Value Assessment. American Society of Clinical Oncology educational book
American Society of Clinical Oncology Annual Meeting. 2019;39:374-87.

[19] Arshad Z, Smith J, Roberts M, Lee WH, Davies B, Bure K, et al. Open Access Could Transform Drug Discovery: A Case Study of JQ1. Expert Opin. Drug Discov. 2016;11(3):321-32.

[20] Di Resta C, Galbiati S, Carrera P, Ferrari M. Next-generation sequencing approach for the diagnosis of human diseases: open challenges and new opportunities. Ejifcc. 2018;29(1):4-14.

[21] Yadav NK, Shukla P, Omer A, Pareek S, Srivastava AK, Bansode FW, et al. Next generation sequencing: potential and application in drug discovery. Scient. World J. 2014;2014:802437.

[22] Loewe RP, Nelson PJ. Microarray bioinformatics. Methods Mol. Biol. 2011;671:295-320.

[23] Carrara L, Lavezzi SM, Borella E, De Nicolao G, Magni P, Poggesi I. Current mathematical models for cancer drug discovery. Expert Opin. Drug Discov. 2017;12(8):785-99.

[24] Dong J, Bao J, Feng R, Zhao Z, Lu Q, Wang G, et al. Circulating microRNAs: a novel potential biomarker for diagnosing acute aortic dissection. Scient. Rep. 2017;7(1):12784.

[25] Shukla AK, Tripathi D. Identification of potential biomarkers on microarray data using distributed gene selection approach. Math. Biosci. 2019;315:108230.

[26] Tikhonov A, Smoldovskaya O, Feyzkhanova G, Kushlinskii N, Rubina A. Glycan-specific antibodies as potential cancer biomarkers: a focus on microarray applications. Clin. Chem. Lab. Med. 2020;58(10):1611-22.

[27] Duan S, Gong B, Wang P, Huang H, Luo L, Liu F. Novel prognostic biomarkers of gastric cancer based on gene expression microarray: COL12A1, GSTA3, FGA and FGG. Mol. Med. Rep. 2018;18(4):3727-36.

[28] Ciocan-Cartita CA, Jurj A, Zanoaga O, Cojoceaneu R, Pop LA, Moldovan A, et al. New insights in gene expression alteration as effect of doxorubicin drug resistance in triple negative breast cancer cells. J. Exp. Clin. Cancer Res. 2020;39(1):241.

[29] Lajos R, Braicu C, Jurj A, Chira S, Cojoceaneu-Petric R, Pileczki V, et al. A miRNAs profile evolution of triple negative breast cancer cells in the presence of a possible adjuvant therapy and senescence inducer. J. BUON 2018;23(3):692-705.

[30] Chen J, Goodchild TT, Brott BC, Li J, King SB, 3rd, Chronos N, et al. Microarray applications in occlusive vascular disease. Cardiovas. Hematol. Agents Med. Chem. 2011;9(2):84-94.

[31] Yoo SM, Choi JH, Lee SY, Yoo NC. Applications of DNA microarray in disease diagnostics. J. Microbiol. Biotech. 2009;19(7):635-46.

[32] Marzancola MG, Sedighi A, Li PC. DNA Microarray-Based Diagnostics. Methods Mol. Biol. 2016;1368:161-78.

[33] Patel A, Cheung SW. Application of DNA Microarray to Clinical Diagnostics. Methods Mol. Boil. 2016;1368:111-32.

[34] Ling H, Pickard K, Ivan C, Isella C, Ikuo M, Mitter R, et al. The clinical and biological significance of MIR-224 expression in colorectal cancer metastasis. Gut. 2016;65(6):977-89.

[35] Pease AC, Solas D, Sullivan EJ, Cronin MT, Holmes CP, Fodor SP. Light-generated oligonucleotide arrays for rapid DNA sequence analysis. Proc. Natl. Acad. Sci. U.S.A. 1994;91(11):5022-6.

[36] Held GA, Grinstein G, Tu Y. Relationship between gene expression
and observed intensities in DNA microarrays—a modeling study. Nucleic Acids Res. 2006;34(9):e70.

[37] Oberthuer A, Juraeva D, Li L, Kahlert Y, Westermann F, Eils R, et al. Comparison of performance of one-color and two-color gene-expression analyses in predicting clinical endpoints of neuroblastoma patients. Pharmacog. J. 2010;10(4):258-66.

[38] Rando O. Nucleic Acid Platform Technologies. Cold Spring Harbor protocols. 2019;2019(9).

[39] Hardiman G. Microarray platforms—comparisons and contrasts. Pharmacogenomics. 2004;5(5):487-502.

[40] Miller MB, Tang YW. Basic concepts of microarrays and potential applications in clinical microbiology. Clin. Microbiol. Rev. 2009;22(4):611-33.

[41] Grist SM, Nasseri SS, Poon T, Roskelley C, Cheung KC. On-chip clearing of arrays of 3-D cell cultures and micro-tissues. Biomicrofluidics. 2016;10(4):044107.

[42] Bovard D, Sandoz A, Luettich K, Frentzel S, Iskandar A, Marescotti D, et al. A lung/liver-on-a-chip platform for acute and chronic toxicity studies. Lab on a chip. 2018;18(24):3814-29.

[43] Bumgarner R. Overview of DNA microarrays: types, applications, and their future. Curr. Protocol. Mol. Biol. 2013;Chapter 22:Unit 22.1.

[44] Zhao S, Niu F, Xu CY, Ye L, Bi GB, Chen L, et al. Microarray and ChIP-seq data analysis revealed changes in p53-mediated transcriptional regulation in Nutlin-3-treated U2OS cells. Mol. Med. Rep. 2015;12(3):4284-90.

[45] Johnston M. Gene chips: array of hope for understanding gene regulation. Curr. Biol: CB. 1998;8(5):R171-4.

[46] Chira S, Raduly L, Braicu C, Jurj A, Cojocneanu-Petric R, Pop L, et al. Premature senescence activation in DLD-1 colorectal cancer cells through adjuvant therapy to induce a miRNA profile modulating cellular death. Exp. Therap. Med. 2018;16(2):1241-9.

[47] Le KQ, Prabhakar BS, Hong WJ, Li LC. Alternative splicing as a biomarker and potential target for drug discovery. Acta Pharmacol. Sinica. 2015;36(10):1212-8.

[48] Leibrand CR, Price DK, Figg WD. Androgen receptor splice variant 7 (AR-V7) and drug efficacy in castration-resistant prostate cancer: Biomarker for treatment selection exclusion or inclusion? Cancer Biol. Therapy. 2016;17(5):467-9.

[49] Zhang T, Karsh LI, Nissenblatt MJ, Canfield SE. Androgen Receptor Splice Variant, AR-V7, as a Biomarker of Resistance to Androgen Axis-Targeted Therapies in Advanced Prostate Cancer. Clin. Genitourinary Cancer. 2020;18(1):1-10.

[50] Huang C-W, Lin Y-T, Ding S-T, Lo L-L, Wang P-H, Lin E-C, et al. Efficient SNP Discovery by Combining Microarray and Lab-on-a-Chip Data for Animal Breeding and Selection. Microarrays. 2015;4(4):570-95.

[51] Ji Y, Mishra RK, Davuluri RV. In silico analysis of alternative splicing on drug-target gene interactions. Sci. Rep. 2020;10(1):134.

[52] Cojocneanu R, Braicu C, Raduly L, Jurj A, Zanoaga O, Magdo L, et al. Plasma and Tissue Specific miRNA Expression Pattern and Functional Analysis Associated to Colorectal Cancer Patients. Cancers. 2020;12(4).

[53] Finan C, Gaulton A, Kruger FA, Lumbers RT, Shah T, Engmann J, et al. The druggable genome and support for target identification and validation in
drug development. Science Translat. Med. 2017;9(383).

[54] Roden DM, McLeod HL, Relling MV, Williams MS, Mensah GA, Peterson JF, et al. Pharmacogenomics. Lancet. 2019; 394(10197):521-32.

[55] Baldwin DA, Sarnowski CP, Reddy SA, Blair IA, Clapper M, Lazarus P, et al. Development of a genotyping microarray for studying the role of gene-environment interactions in risk for lung cancer. J Biomol Tech. 2013;24(4):198-217.

[56] Dally S, Lemuth K, Kaase M, Rupp S, Knabbe C, Weile J. DNA microarray for genotyping antibiotic resistance determinants in Acinetobacter baumanii clinical isolates. Antimicrob Agents Chemother. 2013;57(10):4761-8.

[57] Neverov AA, Riddell MA, Moss WJ, Volokhov DV, Rota PA, Lowe LE, et al. Genotyping of measles virus in clinical specimens on the basis of oligonucleotide microarray hybridization patterns. J Clin Microbiol. 2006;44(10):3752-9.

[58] Shinawi M, Cheung SW. The array CGH and its clinical applications. Drug Discov. Today. 2008;13(17-18):760-70.

[59] Bejjani BA, Shaffer LG. Application of array-based comparative genomic hybridization to clinical diagnostics. The Journal of molecular diagnostics : J. Mol. Diagnost. 2006;8(5):528-33.

[60] Yang HL, Zhu YZ, Qin JH, He P, Jiang XC, Zhao GP, et al. In silico and microarray-based genomic approaches to identifying potential vaccine candidates against Leptospira interrogans. BMC Genomics. 2006;7:293.

[61] Hindson BJ, Ness KD, Masquelier DA, Belgrader P, Heredia NJ, Makarewicz AJ, et al. High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Anal. Chem. 2011;83(22):8604-10.

[62] Hindson CM, Chevillet JR, Briggs HA, Gallichotte EN, Ruf IK, Hindson BJ, et al. Absolute quantification by droplet digital PCR versus analog real-time PCR. Nat. Methods. 2013;10(10):1003-5.

[63] Galbiati S, Damin F, Ferraro L, Soriani N, Burgio V, Ronzoni M, et al. Microarray Approach Combined with ddPCR: An Useful Pipeline for the Detection and Quantification of Circulating Tumour dna Mutations. Cells. 2019;8(8).

[64] Gelsomino L, Gu G, Rechoum Y, Beyer AR, Pejerrey SM, Tsimelzon A, et al. ESR1 mutations affect anti-proliferative responses to tamoxifen through enhanced cross-talk with IGF signaling. Breast Cancer Res. Treat. 2016;157(2):253-65.

[65] Sivarajah S, Kostiuk M, Lindsay C, Puttagunta L, O’Connell DA, Harris J, et al. EGFR as a biomarker of smoking status and survival in oropharyngeal squamous cell carcinoma. J. Otolaryngol. 2019;48(1):1.

[66] Hall SM, Coulter SJ, Knudsen GA, Sanders JM, Birnbaum LS. Gene expression changes in immune response pathways following oral administration of tetrabromobisphenol A (TBBPA) in female Wistar Han rats. Toxicol. Lett. 2017;272:68-74.

[67] Vejdovszky K, Sack M, Jarolim K, Aichinger G, Somoza MM, Marko D. In vitro combinatory effects of the Alternaria mycotoxins alternariol and altertoxin II and potentially involved miRNAs. Toxicol. Lett. 2017; 267:45-52.

[68] Cho SY, Oh Y, Jeong EM, Park S, Lee D, Wang X, et al. Amplification of transglutaminase 2 enhances tumor-promoting inflammation in gastric cancers. Exp. Mol. Med. 2020;52(5): 854-64.
[69] Dang CV, Reddy EP, Shokat KM, Soucek L. Drugging the ‘undruggable’ cancer targets. Nat. Rev. Cancer. 2017;17(8):502-8.

[70] Ray D, Cuneo KC, Rehentulla A, Lawrence TS, Nyati MK. Inducing Oncoprotein Degradation to Improve Targeted Cancer Therapy. Neoplasia (New York, NY). 2015;17(9):697-703.

[71] Hallin J, Engstrom LD, Hargis L, Calinisan A, Aranda R, Briere DM, et al. The KRAS(G12C) Inhibitor MRTX849 Provides Insight toward Therapeutic Susceptibility of KRAS-Mutant Cancers in Mouse Models and Patients. Cancer discovery. 2020;10(1):54-71.

[72] Cox AD, Fesik SW, Kimmelman AC, Luo J, Der CJ. Drugging the undruggable RAS: Mission possible? Nat. Rev. Drug Discovery. 2014;13(11):828-51.

[73] Moore AR, Rosenberg SC, McCormick F, Malek S. RAS-targeted therapies: is the undruggable drugged? Nat. Rev. Drug Discovery. 2020;19(8):533-52.

[74] Allen-Petersen BL, Sears RC. Mission Possible: Advances in MYC Therapeutic Targeting in Cancer. BioDrugs. 2019;33(5):539-53.

[75] Duffy MJ, Synnott NC, Crown J. Mutant p53 as a target for cancer treatment. Eur. J. Cancer. 2017;83:258-65.

[76] Zhang D, Zhang G, Hu X, Wu L, Feng Y, He S, et al. Oncogenic RAS Regulates Long Noncoding RNA Orilnc1 in Human Cancer. Cancer Res. 2017;77(14):3745-57.

[77] Tao J, Zhang R, Singh S, Poddar M, Xu E, Oertel M, et al. Targeting β-catenin in hepatocellular cancers induced by coexpression of mutant β-catenin and K-Ras in mice. Hepatology. 2017;65(5):1581-99.

[78] Kant R, Yen CH, Hung JH, Lu CK, Tung CY, Chang PC, et al. Induction of GNMT by 1,2,3,4,6-penta-O-galloyl-beta-D-glucopyranoside through proteasome-independent MYC downregulation in hepatocellular carcinoma. Scient. Rep. 2019;9(1):1968.

[79] Ye L, Pan J, Liang M, Pasha MA, Shen X, D’Souza SS, et al. A critical role for c-Myc in group 2 innate lymphoid cell activation. Allergy. 2020;75(4):841-52.

[80] Xu J, Chen Y, Huo D, Khramtsov A, Khramtsova G, Zhang C, et al. β-catenin regulates c-Myc and CDKN1A expression in breast cancer cells. Molecular carcinogenesis. 2016;55(5):431-9.

[81] Charni M, Molchadsky A, Goldstein I, Solomon H, Tal P, Goldfinger N, et al. Novel p53 target genes secreted by the liver are involved in non-cell-autonomous regulation. Cell Death Diff. 2016;23(3):509-20.

[82] Koyama R, Tamura M, Nakagaki T, Ohashi T, Idogawa M, Suzuki H, et al. Identification and characterization of a metastatic suppressor BRMS1L as a target gene of p53. Cancer Sci. 2017;108(12):2413-21.

[83] Lee CH, Macgregor PF. Using microarrays to predict resistance to chemotherapy in cancer patients. Pharmacogenomics. 2004;5(6):611-25.

[84] Xu C, Yu Y, Ding F. Microarray analysis of circular RNA expression profiles associated with gemcitabine resistance in pancreatic cancer cells. Oncol. Rep. 2018;40(1):395-404.

[85] Lu L, Wu M, Lu Y, Zhao Z, Liu T, Fu W, et al. MicroRNA-424 regulates cisplatin resistance of gastric cancer by targeting SMURF1 based on GEO database and primary validation in human gastric cancer tissues. Oncol. Targ. Therapy. 2019;12:7623-36.

[86] Januchowski R, Zawierucha P, Ruciński M, Zabel M. Microarray-based
detection and expression analysis of extracellular matrix proteins in drug-resistant ovarian cancer cell lines. Oncology Rep. 2014;32(5):1981-90.

[87] Januchowski R, Sterzyńska K, Zawierucha P, Ruciński M, Świerczewska M, Partyka M, et al. Microarray-based detection and expression analysis of new genes associated with drug resistance in ovarian cancer cell lines. Oncotarget. 2017;8(30):49944-58.

[88] Jurj A, Pop LA, Zanoaga O, Ciocan-Cârtiţă CA, Cojocneanu R, Moldovan C, et al. New Insights in Gene Expression Alteration as Effect of Paclitaxel Drug Resistance in Triple Negative Breast Cancer Cells. Cellular Physiol. Biochem. 2020;54(4):648-64.

[89] You S, Gao L. Identification of NMU as a potential gene conferring alectinib resistance in non-small cell lung cancer based on bioinformatics analyses. Gene. 2018;678:137-42.

[90] Vert A, Castro J, Ribó M, Vilanova M, Benito A. Transcriptional profiling of NCI/ADR-RES cells unveils a complex network of signaling pathways and molecular mechanisms of drug resistance. OncoTargets Therapy. 2018;11:221-37.

[91] Tovar V, Cornella H, Moeini A, Vidal S, Hoshida Y, Sia D, et al. Tumour initiating cells and IGF/FGF signalling contribute to sorafenib resistance in hepatocellular carcinoma. Gut. 2017;66(3):530-40.

[92] Card R, Zhang J, Das P, Cook C, Woodford N, Anjum MF. Evaluation of an expanded microarray for detecting antibiotic resistance genes in a broad range of gram-negative bacterial pathogens. Antimicrobial Agents Chemother. 2013;57(1):458-65.

[93] Frye JG, Lindsey RL, Rondeau G, Porwollik S, Long F, McClelland M, et al. Development of a DNA microarray to detect antimicrobial resistance genes identified in the National Center for Biotechnology Information database. Microbial Drug Resist. 2010;16(1):9-19.

[94] Song Y, Dou F, Zhou Z, Yang N, Zhong J, Pan J, et al. Microarray-Based Detection and Clinical Evaluation for Helicobacter pylori Resistance to Clarithromycin or Levofloxacin and the Genotype of CYP2C19 in 1083 Patients. BioMed Res. Intl. 2018;2018:2684836.

[95] Azizi M, Zaferani M, Dogan B, Zhang S, Simpson KW, Abasspourrad A. Nanoliter-Sized Microchamber/Microarray Microfluidic Platform for Antibiotic Susceptibility Testing. Anal. Chem. 2018;90(24):14137-44.

[96] Charnock C, Samuelsen Ø, Nordlie AL, Hjeltnes B. Use of a Commercially Available Microarray to Characterize Antibiotic-Resistant Clinical Isolates of Klebsiella pneumoniae. Current Microbiol. 2018;75(2):163-72.

[97] Abulwerdi FA, Xu W, Ageeli AA, Yonkunas MJ, Arun G, Nam H, et al. Selective Small-Molecule Targeting of a Triple Helix Encoded by the Long Noncoding RNA, MALAT1. ACS Chem. Biol. 2019;14(2):223-35.

[98] Ursu A, Vézina-Dawod S, Disney MD. Methods to identify and optimize small molecules interacting with RNA (SMIRNAs). Drug Discov. Today. 2019;24(10):2002-16.

[99] Hafeez H, Laurent K, Xiaohui L, Gogce C, Jonas B, Daniel A, et al. Design of a Small Molecule That Stimulates VEGFA Informed from an Expanded Encyclopedia of RNA Fold-Small Molecule Interactions Add - ChemRxiv.

[100] Xu F, Wu J, Wang S, Durmus NG, Gurkan UA, Demirci U. Micro-engineering methods for cell-based microarrays and high-throughput
drug-screening applications. Biofabrication. 2011;3(3):034101.

[101] Bailey SN, Sabatini DM, Stockwell BR. Microarrays of small molecules embedded in biodegradable polymers for use in mammalian cell-based screens. Proc. Natl. Acad. Sci. U.S.A. 2004;101(46):16144-9.

[102] Mateen R, Ali MM, Hoare T. A printable hydrogel microarray for drug screening avoids false positives associated with promiscuous aggregating inhibitors. Nat. Commun. 2018;9(1):602.

[103] Ooko E, Saeed ME, Kadioglu O, Sarvi S, Colak M, Elmasaoudi K, et al. Artemisinin derivatives induce iron-dependent cell death (ferroptosis) in tumor cells. Phytomedicine. 2015;22(11):1045-54.

[104] Liao P, Li W, Liu R, Teer JK, Xu B, Zhang W, et al. Genome-scale analysis identifies SERPINE1 and SPARC as diagnostic and prognostic biomarkers in gastric cancer. OncoTargets Therapy. 2018;11:6969-80.

[105] Seo EJ, Saeed M, Law BY, Wu AG, Kadioglu O, Greten HJ, et al. Pharmacogenomics of Scopoletin in Tumor Cells. Molecules (Basel, Switzerland). 2016;21(4):496.

[106] Zhu QN, Renaud H, Guo Y. Bioinformatic-based identification of miR-542-5p as a predictive biomarker in breast cancer therapy. Hereditas. 2018;155:17.

[107] Gao YF, Zhu T, Mao CX, Liu ZX, Wang ZB, Mao XY, et al. PPIC, EMP3 and CHI3L1 Are Novel Prognostic Markers for High Grade Glioma. Int. J. Mol. Sci. 2016;17(11).

[108] Zambrano T, Hirata RDC, Hirata MH, Cerda Á, Salazar LA. Altered microRNAome Profiling in Statin-Induced HepG2 Cells: A Pilot Study Identifying Potential new Biomarkers Involved in Lipid-Lowering Treatment. Cardiovasc Drugs Therapy. 2015;29(6):509-18.

[109] Fujita K, Nonomura N. Urinary biomarkers of prostate cancer. Int. J. Urology. 2018;25(9):770-9.

[110] Wu J, Wheeldon I, Guo Y, Lu T, Du Y, Wang B, et al. A sandwiched microarray platform for benchtop cell-based high throughput screening. Biomaterials. 2011;32(3):841-8.

[111] Dalerba P, Sahoo D, Paik S, Guo X, Yothers G, Song N, et al. CDX2 as a Prognostic Biomarker in Stage II and Stage III Colon Cancer. The New England journal of medicine. 2016;374(3):211-22.

[112] Lin C, Zhang S, Wang Y, Wang Y, Nice E, Guo C, et al. Functional Role of a Novel Long Noncoding RNA TTN-AS1 in Esophageal Squamous Cell Carcinoma Progression and Metastasis. Clin. Cancer Res. 2018;24(2):486-98.

[113] Carstens MR, Fisher RC, Acharya AP, Butterworth EA, Scott E, Huang EH, et al. Drug-eluting microarrays to identify effective chemotherapeutic combinations targeting patient-derived cancer stem cells. Proc. Natl. Acad. Sci. U.S.A. 2015;112(28):8732-7.

[114] Ding CH, Yin C, Chen SJ, Wen LZ, Ding K, Lei SJ, et al. The HNF1α-regulated lncRNA HNF1A-AS1 reverses the malignancy of hepatocellular carcinoma by enhancing the phosphatase activity of SHP-1. Mol. Cancer. 2018;17(1):63.

[115] Kudou M, Shiozaki A, Yamazato Y, Katsurahara K, Kosuga T, Shoda K, et al. The expression and role of TRPV2 in esophageal squamous cell carcinoma. Sci. Rep. 2019;9(1):16055.

[116] Na Z, Pan S, Uttamchandani M, Yao SQ. Protein-Protein Interaction Inhibitors of BRCA1 Discoveried Using...
Small Molecule Microarrays. Methods Mol. Biol. 2017;1518:139-56.

[117] Lin LC, Kuo TT, Chang HY, Liu WS, Hsia SM, Huang TC. Manzamine A Exerts Anticancer Activity against Human Colorectal Cancer Cells. Marine drugs. 2018;16(8).

[118] Li L, Gao R, Yu Y, Kaul Z, Wang J, Kalra RS, et al. Tumor suppressor activity of miR-451: Identification of CARF as a new target. Sci. Rep. 2018;8(1):375.

[119] Sriram K, Wiley SZ, Moyung K, Gorr MW, Salmerón C, Marucut J, et al. Detection and Quantification of GPCR mRNA: An Assessment and Implications of Data from High-Content Methods. ACS omega. 2019;4(16):17048-59.

[120] Zhao B, Zhao Y, Sun Y, Niu H, Sheng L, Huang D, et al. Alterations in mRNA profiles of trastuzumab-resistant Her-2-positive breast cancer. Mol. Med. Rep. 2018;18(1):139-46.

[121] Zhou Y, Zou H, Yau C, Zhao L, Hall SC, Drummond DC, et al. Discovery of internalizing antibodies to basal breast cancer cells. Protein Eng. Design Sel. 2018;31(1):17-28.

[122] Qu T, Li YP, Li XH, Chen Y. Identification of potential biomarkers and drugs for papillary thyroid cancer based on gene expression profile analysis. Mol. Med. Rep. 2016;14(6):5041-8.

[123] Nguyen MT, Ho-Huynh TD. Nam Dia long, a Vietnamese folk formula, induces apoptosis in MCF-7 cells through various mechanisms of action. BMC Compl. Alt. Med. 2017;17(1):522.

[124] Yang PM, Lin LS, Liu TP. Sorafenib Inhibits Ribonucleotide Reductase Regulatory Subunit M2 (RRM2) in Hepatocellular Carcinoma Cells. Biomolecules. 2020;10(1).

[125] Chen CH, Liu YM, Pan SL, Liu YR, Liou JP, Yen Y. Trichlorobenzene-substituted azaaryl compounds as novel FGFR inhibitors exhibiting potent antitumor activity in bladder cancer cells in vitro and in vivo. Oncotarget. 2016;7(18):26374-87.

[126] Ayyar VS, Sukumaran S, DuBois DC, Almon RR, Jusko WJ. Modeling Corticosteroid Pharmacogenomics and Proteomics in Rat Liver. J. Pharm. Exp. Therap. 2018;367(1):168-83.

[127] Hongkaew Y, Medhasi S, Pasomsub E, Ngamsamut N, Puangpetch A, Vanwong N, et al. UGT1A1 polymorphisms associated with prolactin response in risperidone-treated children and adolescents with autism spectrum disorder. Pharmaco-genomics J. 2018;18(6):740-8.

[128] Eugene AR, Masiak J, Eugene B. Predicting lithium treatment response in bipolar patients using gender-specific gene expression biomarkers and machine learning. F1000Research. 2018;7:474.

[129] Faiz A, Donovan C, Nieuwenhuis MA, van den Berge M, Postma DS, Yao S, et al. Latrophilin receptors: novel bronchodilator targets in asthma. Thorax. 2017;72(1):74-82.

[130] Katsumo S, Tsujimoto G. Genome medicine promised by microarray technology. Expert Rev. Mol. Diagno. 2001;1(4):377-82.

[131] Lamb J, Crawford ED, Peck D, Modell JW, Blat IC, Wrobel MJ, et al. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. Science. 2006;313(5795):1929-35.

[132] Xiong Y, Walker K, Min X, Hale C, Tran T, Komorowski R, et al. Long-acting MIC-1/GDF15 molecules to treat obesity: Evidence from mice to monkeys. Science Transl. Med. 2017;9(412).
[133] Greetham D, Lappin DF, Rajendran R, O’Donnell L, Sherry L, Ramage G, et al. The application of phenotypic microarray analysis to anti-fungal drug development. J. Microbiol. Methods. 2017;134:35-7.

[134] Broszeit F, Tzarum N, Zhu X, Nemanichvili N, Eggink D, Leenders T, et al. N-Glycolyneuraminic Acid as a Receptor for Influenza A Viruses. Cell Rep. 2019;27(11):3284-94.e6.

[135] Stencel-Baerenwald JE, Reiss K, Reiter DM, Stehle T, Dermody TS. The sweet spot: defining virus- sialic acid interactions. Nat. Rev. Microbiol. 2014;12(11):739-49.

[136] Li O, Geng X, Ma Q, Wang W, Liu R, Yin Z, et al. Gene Microarray Integrated with High-Throughput Proteomics for the Discovery of Transthyretin in Rhabdomyolysis-Induced Acute Kidney Injury. Cell. Physiol. Biochem. 2017;43(4):1673-88.

[137] Wilk G, Braun R. regQTLs: Single nucleotide polymorphisms that modulate microRNA regulation of gene expression in tumors. PLoS Genet. 2018;14(12):e1007837.

[138] Hiono T, Matsuda A, Wagatsuma T, Okamatsu M, Sakoda Y, Kuno A. Lectin microarray analyses reveal host cell-specific glycan profiles of the hemagglutinins of influenza A viruses. Virology. 2019;527:132-40.

[139] Sabalza M, Barber CA, Abrams WR, Montagna R, Malamud D. Zika Virus Specific Diagnostic Epitope Discovery. J. Visual. Exp. 2017(130).

[140] Ma C, Lv Q, Teng S, Yu Y, Niu K, Yi C. Identifying key genes in rheumatoid arthritis by weighted gene co-expression network analysis. Int. J. Rheumatic Dis. 2017;20(8):971-9.

[141] Song J, Kim D, Hong J, Kim GW, Jung J, Park S, et al. Meta-Analysis of Polymyositis and Dermatomyositis Microarray Data Reveals Novel Genetic Biomarkers. Genes. 2019;10(11).

[142] Yang PM, Hong YH, Hsu KC, Liu TP. p38α/SIP/SREBP2 activation by the SAM-competitive EZH2 inhibitor GSK343 limits its antitumor activity but creates a druggable vulnerability in hepatocellular carcinoma. Amer. J. Cancer Res. 2019;9(10):2120-39.

[143] Muhammad SA, Raza W, Nguyen T, Bai B, Wu X, Chen J. Cellular Signaling Pathways in Insulin Resistance-Systems Biology Analyses of Microarray Dataset Reveals New Drug Target Gene Signatures of Type 2 Diabetes Mellitus. Front. Physiol. 2017;8:13.

[144] Elmessaoudi-Idrissi M, Windisch MP, Kettani A, Altawalah H, Pineau P, Benjelloun S, et al. An Integrative Gene Expression Microarray Meta-analysis Identifies Host Factors and Key Signatures Involved in Hepatitis B Virus Infection. Infect. Disord. Drug Targ. 2020;20(5):698-707.

[145] Rabinowitz JA, Lainson JC, Johnston SA, Diehnelt CW. Non-natural amino acid peptide microarrays to discover Ebola virus glycoprotein ligands. Chem. Commun. 2018;54(12):1417-20.

[146] Kusi-Appiah AE, Lowry TW, Darrow EM, Wilson KA, Chadwick BP, Davidson MW, et al. Quantitative dose-response curves from subcellular lipid multilayer microarrays. Lab on a chip. 2015;15(16):3397-404.

[147] Fu J. Microarray Selection of Cooperative Peptides for Modulating Enzyme Activities. Microarrays. 2017;6(2).

[148] Pateisky P, Pils D, Szabo L, Kuessel L, Husslein H, Schmitz A, et al. hsav-miRNA-154-5p expression in plasma of endometriosis patients is a potential diagnostic marker for the disease. Reproduct. Biomed. Online. 2018;37(4):449-66.
[149] Jia X, Yu H, Zhang H, Si Y, Tian D, Zhao X, et al. Integrated analysis of different microarray studies to identify candidate genes in type 1 diabetes. J. Diabetes. 2017;9(2):149-57.

[150] Yen CC, Chen SC, Hung GY, Wu PK, Chua WY, Lin YC, et al. Expression profile-driven discovery of AURKA as a treatment target for liposarcoma. Int. J. Oncol. 2019;55(4):938-48.

[151] Bi BL, Wang HJ, Bian H, Tian ZT. Identification of therapeutic targets of ischemic stroke with DNA microarray. Eur. Rev. Med. Pharmacol. 2015;19(21):4012-9.

[152] Bae N, Viviano M, Su X, Lv J, Cheng D, Sagum C, et al. Developing Spindlin1 small-molecule inhibitors by using protein microarrays. Nat. Chem. Biol. 2017;13(7):750-6.

[153] Nguyen BN, Okuno Y, Ajiro M, Iida K, Denawa M, Yamamoto M, et al. Retinoid derivative Tp80 exhibits anti-hepatitis C virus activity through restoration of GI-GPx expression. J. Med. Virol. 2017;89(7):1224-34.

[154] Zlotkowski K, Hewitt WM, Sinniah RS, Tropea JE, Needle D, Lountos GT, et al. A Small-Molecule Microarray Approach for the Identification of E2 Enzyme Inhibitors in Ubiquitin-Like Conjugation Pathways. SLAS Discov. 2017;22(6):760-6.

[155] Sun AG, Lin AQ, Huang SY, Huo D, Cong CH. Identification of potential drugs for Parkinson’s disease based on a sub-pathway method. Int. J. Neurosci. 2016;126(4):318-25.

[156] Sun K, Xie Z, Wang J, Ling M, Li Y, Qiu C. Bioinformatics-based study to detect chemical compounds that show potential as treatments for pulmonary thromboembolism. Int. J. Mol. Med. 2019;43(1):276-84.

[157] Jiang H, Wu FR, Liu J, Qin XJ, Jiang NN, Li WP. Effect of astragalosides on long non-coding RNA expression profiles in rats with adjuvant-induced arthritis. Int. J. Mol. Med. 2019;44(4):1344-56.

[158] Yoshinaga T, Uwabe K, Naito S, Higashino K, Nakano T, Numata Y, et al. AM251 Suppresses Epithelial-Mesenchymal Transition of Renal Tubular Epithelial Cells. PloS one. 2016;11(12):e0167848.

[159] Iwanowycz S, Wang J, Altomare D, Hui Y, Fan D. Emodin Bidirectionally Modulates Macrophage Polarization and Epigenetically Regulates Macrophage Memory. J. Biol. Chem. 2016;291(22):11491-503.

[160] Woods MW, Zahoor MA, Dizzell S, Verschoor CP, Kaushic C. Medroxyprogesterone acetate-treated human, primary endometrial epithelial cells reveal unique gene expression signature linked to innate immunity and HIV-1 susceptibility. Amer. J. Rep. Immunol. 2018;79(1).

[161] Ye J, Xu B, Fan B, Zhang J, Yuan F, Chen Y, et al. Discovery of Selenocysteine as a Potential Nanomedicine Promotes Cartilage Regeneration With Enhanced Immune Response by Text Mining and Biomedical Databases. Front. Pharmacol. 2020;11:1138.

[162] Behjati S, Tarpey PS. What is next generation sequencing? Arch. Disease Childhood Ed. Pract. Ed. 2013;98(6):236-8.

[163] Dulanto Chiang A, Dekker JP. From the Pipeline to the Bedside: Advances and Challenges in Clinical Metagenomics. J. Infect. Dis. 2020;221(Supplement_3):S331-s40.

[164] Lucan C, Pop LA, Florian A, Pileczki V, Petrushev B, Dima D, et al. HLA Genotyping using Next Generation
Sequencing. Roman. J. Internal Med. 2016;54(2):98-104.

[165] Tomuleasa C, Selicean C, Cismas S, Jurj A, Marian M, Dima D, et al. Minimal residual disease in chronic lymphocytic leukemia: A consensus paper that presents the clinical impact of the presently available laboratory approaches. Crit. Rev. Clin. Lab. Sci. 2018;55(5):329-45.

[166] Woollard PM, Mehta NA, Vamathivan JJ, Van Horn S, Bonde BK, Dow DJ. The application of next-generation sequencing technologies to drug discovery and development. Drug Discov. Today. 2011;16(11-12):512-9.

[167] Braicu C, Buiga R, Cojocneanu R, Buse M, Raduly L, Pop LA, et al. Connecting the dots between different networks: miRNAs associated with bladder cancer risk and progression. J. Exp. Clin. Cancer Res. 2019;38(1):433.

[168] Ciocan-Cărtiță CA, Jurj A, Raduly L, Cojocneanu R, Moldovan A, Pileczki V, et al. New perspectives in triple-negative breast cancer therapy based on treatments with TGFβ1 siRNA and doxorubicin. Mol. Cell. Biochem. 2020;475(1-2):285-99.

[169] Rubin A, Salzberg AC, Imamura Y, Gravitishvilli A, Tombran-Tink J. Identification of novel targets of diabetic nephropathy and PEDF peptide treatment using RNA-seq. BMC genomics. 2016;17(1):936.

[170] Ng SB, Buckingham KJ, Lee C, Bigham AW, Tabor HK, Dent KM, et al. Exome sequencing identifies the cause of a mendelian disorder. Nat. Genet. 2010;42(1):30-5.

[171] Sas V, Pasca S, Jurj A, Pop L, Muramatsu H, Ono H, et al. MicroRNA-155-5p Plays a Critical Role in Transient Leukemia of Down Syndrome by Targeting Tumor Necrosis Factor Receptor Superfamily Members. Cell. Physiol. Biochem. 2020;54(5):994-1012.

[172] Wang Z, Li Y, Hou B, Pronobis MI, Wang M, Wang Y, et al. An array of 60,000 antibodies for proteome-scale antibody generation and target discovery. Science advances. 2020;6(11):eaax2271.

[173] Petric RC, Pop LA, Jurj A, Raduly L, Dumitrascu D, Dragos N, et al. Next generation sequencing applications for breast cancer research. Clujul Med. 2015;88(3):278-87.

[174] NCCN Clinical Practice Guidelines in Oncology: Non-Small Cell Lung Cancer [Internet]. NCCN. 2020 [cited Accessed on 17.08.2020]. Available from: https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf.

[175] NCCN Clinical Practice Guidelines in Oncology. Colon Cancer [Internet]. NCCn. 2020 [cited 17 August 2020]. Available from: https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf.

[176] NCCN Clinical Practice Guidelines in Oncology. Breast and ovarian cancer [Internet]. NCCN. 2020 [cited 17 August 2020]. Available from: https://www.nccn.org/professionals/physician_gls/pdf/genetics_bop.pdf.

[177] Treon SP, Xu L, Guerrera ML, Jimenez C, Hunter ZR, Liu X, et al. Genomic Landscape of Waldenström Macroglobulinemia and Its Impact on Treatment Strategies. J. Clin. Oncol. 2020;38(11):1198-208.

[178] Tshibangu-Kabamba E, Ngoma-Kisoko PJ, Tuan VP, Matsumoto T, Akada J, Kido Y, et al. Next-Generation Sequencing of the Whole Bacterial Genome for Tracking Molecular Insight into the Broad-Spectrum Antimicrobial Resistance of Helicobacter pylori Clinical Isolates from the Democratic Republic of Congo. Microorganisms. 2020;8(6).
Butler DJ, Mozsary C, Meydan C, Danko D, Foono J, Rosiene J, et al. Shotgun Transcriptome and Isothermal Profiling of SARS-CoV-2 Infection Reveals Unique Host Responses, Viral Diversification, and Drug Interactions. bioRxiv. 2020;2020.04.20.048066.

Ureshino H, Koarada S, Kamachi K, Yoshimura M, Yokoo M, Kubota Y, et al. Immune dysregulation syndrome with de novo CTLA4 germline mutation responsive to abatacept therapy. Int. J. Hematol. 2020;111(6):897-902.

Schatz S, Falk M, Jóri B, Ramdani HO, Schmidt S, Willing EM, et al. Integration of Tumor Mutation Burden and PD-L1 Testing in Routine Laboratory Diagnostics in Non-Small Cell Lung Cancer. Cancers. 2020;12(6).

Pop LA, Cojocneanu-Petric RM, Pileczki V, Morar-Bolba G, Irimie A, Lazar V, et al. Genetic alterations in sporadic triple negative breast cancer. Breast. 2018;38:30-8.

Klowak JA, El Helou S, Pernica JM, Parker MJ, Surette M, Pinar H, et al. Fast I(n)dentification of Pathogens in Neonates (FINDPATH-N): protocol for a prospective pilot cohort study of next-generation sequencing for pathogen identification in neonates with suspected sepsis. BMJ Paediat. Open. 2020;4(1):e000651.

McConnell L, Houghton O, Stewart P, Gazdova J, Srivastava S, Kim C, et al. A novel next generation sequencing approach to improve sarcoma diagnosis. Modern Pathol. 2020;33(7):1350-9.

Yang F, Anekpuritanang T, Press RD. Clinical Utility of Next-Generation Sequencing in Acute Myeloid Leukemia. Mol. Diagn. Ther. 2020;24(1):1-13.

Dy GK, Nesline MK, Papanicolaou-Sengos A, DePietro P, LeVea CM, Early A, et al. Treatment recommendations to cancer patients in the context of FDA guidance for next generation sequencing. BMC Med. Inform. Decis. Mak. 2019;19(1):14.

Farnaes L, Wilke J, Ryan Loker K, Bradley JS, Cannavino CR, Hong DK, et al. Community-acquired pneumonia in children: cell-free plasma sequencing for diagnosis and management. Microbiol. Infectious Disease. 2019;94(2):188-91.

Farrow E, Rengasamy Venugopalan S, Thiffault I, Saunders C. Examination of rare genetic variants in dental enamel genes: The potential role of next-generation sequencing in primary dental care. Orthodont. Craniofac. Res. 2019;22 Suppl 1:49-55.

Réda M, Richard C, Bertaut A, Niogret J, Collot T, Fumet JD, et al. Implementation and use of whole exome sequencing for metastatic solid cancer. EBioMedicine. 2020;51:102624.

Goodnow RA, Davie CP. Chapter One - DNA-Encoded Library Technology: A Brief Guide to Its Evolution and Impact on Drug Discovery. In: Goodnow RA, editor. Annual Reports in Medicinal Chemistry. 50: Academic Press; 2017. p. 1-15.

Franzini RM, Neri D, Scheuermann J. DNA-encoded chemical libraries: advancing beyond conventional small-molecule libraries. Aco. Chem. Res. 2014;47(4):1247-55.

Goodnow RA, Jr., Dumelin CE, Keefe AD. DNA-encoded chemistry: enabling the deeper sampling of chemical space. Nat. Rev. Drug Discov. 2017;16(2):131-47.

Neri D, Lerner RA. DNA-Encoded Chemical Libraries: A Selection System Based on Endowing Organic Compounds with Amplifiable Information. Annu. Rev. Biochem. 2018;87:479-502.
[194] Salamon H, Klika Škopić M, Jung K, Bugain O, Brunschweiger A. Chemical Biology Probes from Advanced DNA-encoded Libraries. ACS Chem. Biol. 2016;11(2):296-307.

[195] Lemke M, Ravenscroft H, Rueb NJ, Kireev D, Ferraris D, Franzini RM. Integrating DNA-encoded chemical libraries with virtual combinatorial library screening: Optimizing a PARP10 inhibitor. Bioorganic Med. Chem. Lett. 2020;30(19):127464.

[196] Reidenbach AG, Mesleh MF, Casalena D, Vallabh SM, Dahlin JL, Leed AJ, et al. Multimodal small-molecule screening for human prion protein binders. J. Biol. Chem. 2020.

[197] Cuozzo JW, Clark MA, Keefe AD, Kohlmann A, Mulvihill M, Ni H, et al. Novel Autotaxin Inhibitor for the Treatment of Idiopathic Pulmonary Fibrosis: A Clinical Candidate Discovered Using DNA-Encoded Chemistry. J. Med. Chem. 2020;63(14):7840-56.

[198] Dawadi S, Simmons N, Miklossy G, Bohren KM, Faver JC, Ucisik MN, et al. Discovery of potent thrombin inhibitors from a protease-focused DNA-encoded chemical library. Proc. Natl. Acad. Sci. U.S.A. 2020;117(29):16782-9.

[199] Kung PP, Bingham P, Burke BJ, Chen Q, Cheng X, Deng YL, et al. Characterization of Specific N-α-Acetyltransferase 50 (Naa50) Inhibitors Identified Using a DNA Encoded Library. ACS Med. Chem. Lett. 2020;11(6):1175-84.

[200] Arakaki AK, Tian W, Skolnick J. High precision multi-genome scale reannotation of enzyme function by EFICAz. BMC genomics. 2006;7:315.

[201] Verdine GL, Walensky LD. The challenge of drugging undruggable targets in cancer: lessons learned from targeting BCL-2 family members. Clin. Cancer Res. 2007;13(24):7264-70.

[202] Passiglia F, Malapelle U, Del Re M, Righi L, Pagni F, Furlan D, et al. KRAS inhibition in non-small cell lung cancer: Past failures, new findings and upcoming challenges. Eur. J. Cancer. 2020;137:57-68.

[203] Kato S, Okamura R, Sicklick JK, Daniels GA, Hong DS, Goodman A, et al. Prognostic implications of RAS alterations in diverse malignancies and impact of targeted therapies. Int. J. Cancer. 2020;146(12):3450-60.

[204] Duffy MJ, Crown J. Drugging "undruggable" genes for cancer treatment: Are we making progress? Int. J. Cancer. 2020.

[205] Zhou J, Zhao W, Wu J, Lu J, Ding Y, Wu S, et al. Neoantigens Derived from Recurrently Mutated Genes as Potential Immunotherapy Targets for Gastric Cancer. BioMed Res. Int. 2019;2019:8103142.

[206] Duffy DJ, Krstic A, Halasz M, Schwarzl T, Fey D, Iljin K, et al. Integrative omics reveals MYCN as a global suppressor of cellular signalling and enables network-based therapeutic target discovery in neuroblastoma. Oncotarget. 2015;6(41):43182-201.

[207] Ng CK, How KY, Tee KK, Chan KG. Characterization and Transcriptome Studies of Autoinducer Synthase Gene from Multidrug Resistant Acinetobacter baumannii Strain 863. Genes. 2019;10(4).

[208] Subramanian D, Natarajan J. RNA-seq analysis reveals resistome genes and signalling pathway associated with vancomycin-intermediate Staphylococcus aureus. Ind. J. Med. Microbiol. 2019;37(2):173-85.

[209] Chou S. Approach to drug-resistant cytomegalovirus in transplant recipients. Curr. Opin. Infect. Dis. 2015;28(4):293-9.

[210] Port JA, Wallace JC, Griffith WC, Faustman EM. Metagenomic profiling of microbial composition and antibiotic
resistance determinants in Puget Sound. PloS one. 2012;7(10):e48000.

[211] Leprohon P, Fernandez-Prada C, Gazanion É, Monte-Neto R, Ouellette M. Drug resistance analysis by next generation sequencing in Leishmania. Int. J. Parasitol. Drugs Drug Resist. 2015;5(1):26-35.

[212] Lee ER, Parkin N, Jennings C, Brumme CJ, Enns E, Casadellà M, et al. Performance comparison of next generation sequencing analysis pipelines for HIV-1 drug resistance testing. Sci. Rep. 2020;10(1):1634.

[213] Fogel JM, Bonsall D, Cummings V, Bowden R, Golubchik T, de Cesare M, et al. Performance of a high-throughput next-generation sequencing method for analysis of HIV drug resistance and viral load. J. Antimicrob. Chemother. 2020.

[214] Sivay MV, Palumbo PJ, Zhang Y, Cummings V, Guo X, Hamilton EL, et al. HIV drug resistance, phylogenetic analysis, and superinfection among men who have sex with men and transgender women in sub-Saharan Africa: HPTN 075. Clinical Infect. Dis. 2020.

[215] Noguera-Julian M, Lee ER, Shafer RW, Kantor R, Ji H. Dry Panels Supporting External Quality Assessment Programs for Next Generation Sequencing-Based HIV Drug Resistance Testing. Viruses. 2020;12(6).

[216] Ávila-Ríos S, Parkin N, Swanstrom R, Paredes R, Shafer R, Ji H, et al. Next-Generation Sequencing for HIV Drug Resistance Testing: Laboratory, Clinical, and Implementation Considerations. Viruses. 2020;12(6).

[217] Patel MC, Mishin VP, De La Cruz JA, Chesnokov A, Nguyen HT, Wilson MM, et al. Detection of baloxavir resistant influenza A viruses using next generation sequencing and pyrosequencing methods. Antiviral research. 2020;182:104906.

[218] Ye L, Meng F, Mao X, Zhang Y, Wang J, Liu Y, et al. Using next-generation sequencing to analyze Helicobacter pylori clones with different levofloxacin resistances from a patient with eradication failure. Medicine. 2020;99(32):e20761.

[219] Cho WC, Shin YK, Na YS, Ryu MH, Ku JL, Kang YK. The role of novel fusion genes in human GIST cell lines derived from imatinib-resistant GIST patients: A therapeutic potential of fusion gene. Biochem. Biophys. Res. Commun. 2020;529(3):699-706.

[220] Wishart DS, Knox C, Guo AC, Shrivastava S, Hassanali M, Stothard P, et al. DrugBank: a comprehensive resource for in silico drug discovery and exploration. Nucleic Acids Res. 2006;34(Database issue):D668-72.

[221] Wishart DS, Knox C, Guo AC, Cheng D, Shrivastava S, Tzur D, et al. DrugBank: a knowledgebase for drugs, drug actions and drug targets. Nucleic Acids Res. 2008;36(Database issue):D901-6.

[222] Wishart DS. Discovering drug targets through the web. Comparative biochemistry and physiology Part D, Genomics & proteomics. 2007;2(1):9-17.

[223] Chang C, Bahadduri PM, Polli JE, Swaan PW, Ekins S. Rapid identification of P-glycoprotein substrates and inhibitors. Drug Metabol. Disposit. 2006;34(12):1976-84.

[224] Jolivette LJ, Ekins S. Methods for predicting human drug metabolism. Advances in clinical chemistry. 2007;43:131-76.

[225] Li H, Gao Z, Kang L, Zhang H, Yang K, Yu K, et al. TarFisDock: a web server for identifying drug targets with docking approach. Nucleic Acids Res. 2006;34(Web Server issue):W219-24.
[226] Chong CR, Sullivan DJ, Jr. New uses for old drugs. Nature. 2007; 448(7154):645-6.

[227] Jiang T, Kong B, Yan W, Wu C, Jiang M, Xu X, et al. Network Pharmacology to Identify the Pharmacological Mechanisms of a Traditional Chinese Medicine Derived from Trachelospermum jasminoides in Patients with Rheumatoid Arthritis. Med. Sci. Monitor. 2020;26:e922639.

[228] Lokhande KB, Doiphode S, Vyas R, Swamy KV. Molecular docking and simulation studies on SARS-CoV-2 M(pro) reveals Mitoxantrone, Leucovorin, Birinapant, and Dynasore as potent drugs against COVID-19. J. Biomol. Struct. Dynam. 2020:1-12.

[229] Mazzolari A, Gervasoni S, Pedretti A, Fumagalli L, Matucci R, Vistoli G. Repositioning Dequalinium as Potent Muscarinic Allosteric Ligand by Combining Virtual Screening Campaigns and Experimental Binding Assays. Int. J. Mol. Sci. 2020;21(17).

[230] Thomas RE. Optimising Seniors’ Metabolism of Medications and Avoiding Adverse Drug Events Using Data on How Metabolism by Their P450 Enzymes Varies with Ancestry and Drug-Drug and Drug-Drug-Gene Interactions. J. Personal. Med. 2020;10(3).

[231] Xu C, Ke Z, Liu C, Wang Z, Liu D, Zhang L, et al. Systemic in Silico Screening in Drug Discovery for Coronavirus Disease (COVID-19) with an Online Interactive Web Server. J. Chem. Inf. Model. 2020.

[232] Ramachandran B, Jeyakanthan J, Lopes BS. Molecular docking, dynamics and free energy analyses of Acinetobacter baumannii OXA class enzymes with carbapenems investigating their hydrolytic mechanisms. J. Med. Microbiol. 2020.

[233] Khelfaoui H, Harkati D, Saleh BA. Molecular docking, molecular dynamics simulations and reactivity, studies on approved drugs library targeting ACE2 and SARS-CoV-2 binding with ACE2. J. Biomol. Struct. Dynam. 2020:1-17.

[234] Wei TZ, Wang H, Wu XQ, Lu Y, Guan SH, Dong FQ, et al. In Silico Screening of Potential Spike Glycoprotein Inhibitors of SARS-CoV-2 with Drug Repurposing Strategy. Chinese J. Integr. Med. 2020:1-7.

[235] Fabbri C, Kasper S, Zohar J, Souery D, Montgomery S, Albani D, et al. Drug repositioning for treatment-resistant depression: Hypotheses from a pharmacogenomic study. Progress Neuro-psychopharmacol. Biol. Psych. 2020;104:110050.

[236] Lu X, Wu X, Jing L, Tao L, Zhang Y, Huang R, et al. Network Pharmacology Analysis and Experiments Validation of the Inhibitory Effect of JianPi Fu Recipe on Colorectal Cancer LoVo Cells Metastasis and Growth. Evidence-based complementary and alternative medicine : eCAM. 2020;2020:4517483.

[237] Li T, Liu Q, Zhang R, Liao Q, Zhao Y. Identification of prognosis-related genes and construction of multi-regulatory networks in pancreatic cancer microenvironment by bioinformatics analysis. Cancer Cell Int. 2020;20:341.

[238] Fiorucci D, Milletti E, Orofino F, Brizzi A, Mugnaini C, Corelli F. Computational drug repurposing for the identification of SARS-CoV-2 main protease inhibitors. J. Biomol. Struct. Dynam. 2020:1-7.

[239] Ibrahim MAA, Abdelrahman AHM, Hegazy MF. In-silico drug repurposing and molecular dynamics puzzled out potential SARS-CoV-2 main protease inhibitors. J. Biomol. Struct. Dynam. 2020:1-12.
[240] Masoudi-Sobhanzadeh Y, Masoudi-Nejad A. Synthetic repurposing of drugs against hypertension: a datamining method based on association rules and a novel discrete algorithm. BMC Bioinform. 2020;21(1):313.

[241] Huang B, Xiong J, Zhao X, Zheng Y, Zhu N. Network Pharmacology-Based Analysis of the Pharmacological Mechanisms of Aloperine on Cardiovascular Disease. Evidence-based complementary and alternative medicine : eCAM. 2020;2020:5180716.

[242] Zhao Z, Li J, Li H, Yuan Wu NY, Ou-Yang P, Liu S, et al. Integrative Bioinformatics Approaches to Screen Potential Prognostic Immune-Related Genes and Drugs in the Cervical Cancer Microenvironment. Front. Genet. 2020;11:727.

[243] Chandra A, Gurjar V, Ahmed MZ, Alqahtani AS, Qamar I, Singh N. Exploring potential inhibitor of SARS-CoV2 replicase from FDA approved drugs using insilico drug discovery methods. J. Biomol. Struct. Dynam. 2021:1-8.

[244] Gupta Y, Maciorowski D, Zak SE, Jones KA, Kathayat RS, Azizi SA, et al. Bisindolylmaleimide IX: A novel anti-SARS-CoV2 agent targeting viral main protease 3CLpro demonstrated by virtual screening pipeline and in-vitro validation assays. Methods (San Diego, Calif). 2021.

[245] Kwarteng A, Asiedu E, Sakyi SA, Asiedu SO. Targeting the SARS-CoV2 nucleocapsid protein for potential therapeutics using immuno-informatics and structure-based drug discovery techniques. Biomed. Pharmacother. 2020;132:110914.

[246] Mok PL, Koh AE, Farhana A, Alsrhani A, Alam MK, Suresh Kumar S. Computational drug screening against the SARS-CoV-2 Saudi Arabia isolates through a multiple-sequence alignment approach. Saudi J. Biol. Sci. 2021.

[247] Anwar F, Altayb HN, Al-Abbasi FA, Kumar V, Kamal MA. The computational intervention of macrolide antibiotics in the treatment of COVID-19. Curr. Pharmaceut. Design. 2021.

[248] Fiscon G, Conte F, Farina L, Paci P. SAVeRUNNER: A network-based algorithm for drug repurposing and its application to COVID-19. PLoS computational biology. 2021;17(2):e1008686.

[249] Song L, Xiao G, Tang R, Zhang X, Gao Z, Sun S, et al. Next-generation sequencing and RT-PCR to identify a 32-day SARS-CoV-2 carrier. Clinical chemistry and laboratory medicine. 2021.

[250] Papoutsis A, Borody T, Dolai S, Daniels J, Steinberg S, Barrows B, et al. Detection of SARS-CoV-2 from patient fecal samples by whole genome sequencing. Gut pathogens. 2021;13(1):7.

[251] Armero A, Berthet N, Avarre JC. Intra-Host Diversity of SARS-Cov-2 Should Not Be Neglected: Case of the State of Victoria, Australia. Viruses. 2021;13(1).

[252] Charre C, Ginevra C, Sabatier M, Regue H, Destras G, Brun S, et al. Evaluation of NGS-based approaches for SARS-CoV-2 whole genome characterisation. Virus Evol. 2020;6(2):veaa075.

[253] Panzera Y, Ramos N, Frabasile S, Calleros L, Marandino A, Tomás G, et al. A deletion in SARS-CoV-2 ORF7 identified in COVID-19 outbreak in Uruguay. Transbound. Emerg. Dis. 2021.

[254] Tushir S, Kamanna S, Nath SS, Bhat A, Rose S, Aithal AR, et al. Proteo-Genomic Analysis of SARS-CoV-2: A Clinical Landscape of Single-Nucleotide Polymorphisms, COVID-19 Proteome, and Host Responses. J. Proteome Res. 2021.
[255] Gunadi, Wibawa H, Marcellus, Hakim MS, Daniwijaya EW, Rizki LP, et al. Full-length genome characterization and phylogenetic analysis of SARS-CoV-2 virus strains from Yogyakarta and Central Java, Indonesia. PeerJ. 2020;8:e10575.

[256] Wei J, Alfajaro MM, DeWeirdt PC, Hanna RE, Lu-Culligan WJ, Cai WL, et al. Genome-wide CRISPR Screens Reveal Host Factors Critical for SARS-CoV-2 Infection. Cell. 2021;184(1):76-91.e13.