Drug development review

An overview of recent publications featuring Illumina technology

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INTRODUCTION

The development of new therapeutic agents increasingly relies on the use of genomic analysis tools for better understanding of several factors. These factors include the drug's mode of action, predicting its patient-specific efficiency and toxicity, and developing companion diagnostics and combination drugs. Genetic heterogeneity, which becomes increasingly well-understood and utilized with the advent of next-generation sequencing (NGS) technology, is expected to reorient drug discovery strategy. Computational modeling and rapidly developing bioinformatics are playing more important roles in this process, because approaches that consider combinations of genes explain the nature of disease better than single-gene approaches.[1] NGS and microarray provide an end-to-end solution for explaining the role of genetic predisposition, clonal heterogeneity, and drug resistance throughout the whole drug development cycle, from target discovery to clinical trials to drug repurposing.[2 3] Identifying the best treatment strategy based on genomic information is a core goal of "personalized" or "stratified" medicine.[4 5 6] The recognition of the importance of implementing genomic analysis tools in drug development by regulatory agencies, and an increasing implementation of NGS by pharmaceutical companies portends a rapid transformation of the research and development approach in the pharmaceutical industry.[7]

Reviews

Ong F. S., Lin J. C., Das K., Grosu D. S. and Fan J. B. (2013) Translational utility of next-generation sequencing. Genomics 102: 137-139

NGS techniques have opened up new avenues for genomic characterization across many areas of molecular pathology. This review discusses how the latest improvements in accuracy, throughout, and single-cell sequencing techniques have wide-ranging applications for clinical decision-making. The authors give an overview of methods and applications that are expected to become central in the development of genomic medicine.

Pant S., Weiner R. and Marton M. J. (2014) Navigating the rapids: the development of regulated next-generation sequencing-based clinical trial assays and companion diagnostics. Front Oncol 4: 78

Pasic M. D., Samaan S. and Yousef G. M. (2013) Genomic medicine: new frontiers and new challenges. Clin Chem 59: 158-167

Simon R. and Roychowdhury S. (2013) Implementing personalized cancer genomics in clinical trials. Nat Rev Drug Discov 12: 358-369

Dancey J. E., Bedard P. L., Onetto N. and Hudson T. J. (2012) The genetic basis for cancer treatment decisions. Cell 148: 409-420

Woollard P. M., Mehta N. A., Vamathevan J. J., Van Horn S., Bonde B. K., et al. (2011) The application of next-generation sequencing technologies to drug discovery and development. Drug Discov Today 16: 512-519

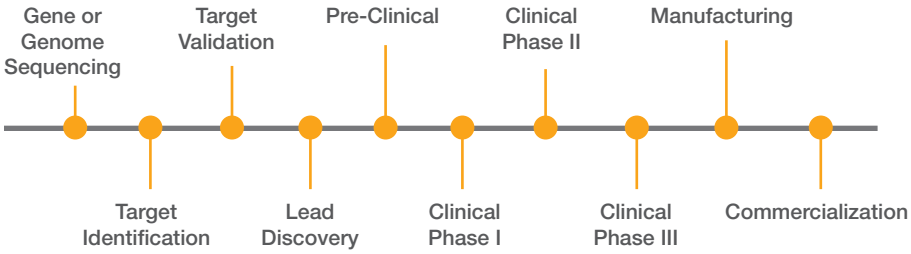
DRUG DEVELOPMENT PROCESS

NGS approaches, with minor adjustments in sample preparation, can generate multiple levels of genomic data essential for the drug development: genomic modifications, transcriptome profiling and quantification, miRNA profiling, epigenetic modifications at the DNA and histone level, RNA-protein interactions, and analysis of the role of the microbiome in drug resistance.[8] NGS is becoming indispensable for diagnosing and controlling the progression of diseases that are highly heterogeneous,[8] caused by a large number of genetic alterations, or have a significant environmental component (complex diseases).[9 10] The average number of driver mutations in most cancers is 2 to 6.[11] In neurological diseases, the number of risk-associated loci can reach thousands,[12] underscoring the need for genome-wide high-throughput screening at all stages of drug development. Identification of clinically relevant mutations remains challenging, as it requires a thorough understanding of simultaneously quantitated gene-gene networks and the genotype-phenotype connection.

"The timing is right to develop a clinical trial and research framework to move future clinical decisions from heuristic to evidence-based decisions."

Dancey et al. 2012

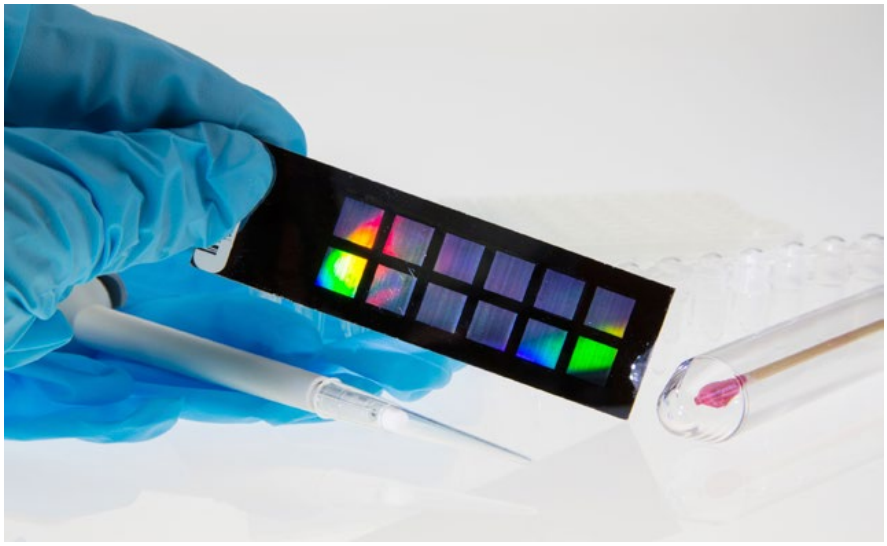
Workflow for Development



NGS can be repeatedly used at multiple phases across this workflow.

Clinical labs across the country have developed hundreds of laboratory-developed tests (LDT) based on NGS,[13] and most of these facilities are Clinical Lab Improvement Amendment (CLIA)-certified[8] –a prerequisite for conducting clinical trial-related studies.[8] NGS-based cancer panels are already being used for triaging patients in clinical trials to identify the most appropriate biomarker-informed course of treatment.[14 15 16] In this age of information, patients also are increasingly willing to invest in personal genomic analysis for information about disease predisposition and for better understanding of diagnosed diseases.

The drug development process can be roughly partitioned into 5 stages: target identification, target validation, preclinical development, clinical development, and postclinical development. The role and perspectives of using Illumina technology at each of these stages is delineated in the following chapters.



Target identification

Target identification is a process of identifying "actionable" mutations, genes, or proteins for targeting via an existing drug or a drug candidate. "Target" is a broad concept encompassing a range of actionable entities, from macromolecules (genes or regulatory nucleic acids and proteins) to molecular functions and pathways.[17] In recent years, differential gene expression studies in normal versus diseased tissues or patients have become a streamlined approach to preliminary target identification, allowing for matching actionable mutations and target genes with existing therapies and drug candidates.[2] Currently, 3 major techniques based on NGS technology are used for target identification (Table 1). In setting out to test a therapeutic hypothesis, a fundamental initial consideration is how to select NGS-techniques to maximize information density within samples in a high-throughput and economical fashion.

Table 1: Application of NGS-Based Techniques for Target Identification.

Technique	Major application	References
Whole-exome sequencing (WES)	Detection of Mendelian diseases	2 18
	Cost-efficient genetic linkage studies (gene-gene interactions are analyzed, and targets for drugs aimed at enhancing or inhibiting those linkages are identified)	2 19
Whole-genome sequencing (WGS)	Hypothesis-free comprehensive interrogation of mutations across copy-number variants (CNVs), indels, single-nucleotide variants (SNVs), and structural variants (SVs).	http://www.ncbi.nlm.nih.gov/pubmed/22549284
RNA sequencing (transcriptome analysis)	Measure expression patterns of thousands of genes simultaneously to provide insight into functional pathways and regulation of biological processes.	Khatoon Z, Figler B, Zhang H, Cheng F. (2014) Introduction to RNA-Seq and its Applications to Drug Discovery and Development. Drug Dev Res 75(5):324-30.

Target identification by NGS not only aids in finding "actionable" gene targets, but also may lead to identification of new driver mutations relevant to the specific disease.[20] Recurrent gene fusions, such as *BCR-ABL* gene fusions in chronic myeloid leukemia (CML) or fibroblast growth factor receptor (FGFR) family fusions in carcinomas,[20] are some of the most prominent examples of such driver mutations.[21]

Target identification processes employ informatics tools (a "system" approach) along with a traditional "molecular" approach.[17] In order to therapeutically exploit a gene, it remains essential to establish the biological role of the gene and/or protein in disease causation and the cognate biochemical pathway. Biological knowledge and pathways, computational tools, and statistical methods are aligned with a goal to identify and prioritize targets in this "data mining" process. Microarray data mining is one of the most common examples: this approach was used to discover the hypermethylated gene encoding insulin-like growth factor-binding protein 3 (IGFBP3)—a biomarker of prostate cancer.[22] Other examples of data mining include proteomic mining, text mining (automatic retrieval of information from different written sources), and chemogenomic mining (high-throughput analysis of cell phenotype of interest by applying libraries of small molecules to a library of cells).[17]

The concept of "genometype," which refers to the genomes in the population with a genetic risk for a specific disease,[23] has recently been introduced. This concept highlights the need for considering the importance of patient stratification early in the drug development process, far in advance of proceeding to clinical trials. Roberts et al. have tested the capacity of WGS to identify individuals with significant risk of developing 24 different diseases, including cancer, stroke, and coronary heart disease. The authors have concluded that over 90% of tested individuals may be clinically predisposed to at least one disease, but these data were limited only to relatively common diseases.[23] The selection of drug targets for validation has continued to shift from an ad hoc process to one in which NGS data offers both breadth and depth of data relevant to multiple clinical needs.

Many oncoproteins are intractable with conventional medicinal chemistry approaches and thus necessitate more nuanced therapeutic approaches. RNA interference (RNAi) screens are becoming a popular way to identify genes involved in oncogenic growth control.[24] MicroRNA (miRNA), small interfering RNA (siRNA), and small hairpin RNA (shRNA) are sensitive indicators of the transformative processes associated with many diseases: changes of their intracellular levels often correlate with the expression levels of potential target genes. Braun et al. have introduced a new technology for trapping regulatory miRNA as drug targets: NGS combined with miRNA trapping by RNA in vitro affinity purification (miTRAP). This technique allows for capturing miRNA with noncanonical binding sites that are otherwise hard to predict using conventional bioinformatics tools.[25]

Another new approach to target identification was proposed by Anders et al. The authors have combined the power of DNA NGS and chromatin immunoprecipitation sequencing (ChIP-Seq) with chemical ligand-affinity capture, in a technique called Chem-Seq[26]. Essentially, this process is a "reverse" target identification approach that allows genome-wide identification of points of interaction of small molecule drugs with DNA or genome-associated proteins.[26] The authors have verified the robustness of this approach by demonstrating binding of a biotinylated derivative of the bromodomain inhibitor JQ1 to the bromodomain and extraterminal domain (BET) protein family members in multiple myeloma cells, both in cell culture and in nuclear lysates.[26] This mapping of gene-drug interactions can be very instrumental in assessing specificity of drugs, individual variability in drug response, and finding alternative applications for drugs and drug candidates.

Reviews

Lechartier B., Rybniker J., Zumla A. and Cole S. T. (2014) Tuberculosis drug discovery in the post-post-genomic era. EMBO Mol Med 6: 158-168

Tuberculosis (TB) is a global health problem. Genome sequencing technologies are promising new avenues for identifying therapeutic interventions. TB drug research in the postgenomic era uses NGS to characterize the genome sequence of *Mycobacterium tuberculosis* to identify resistance mutations and targets, resulting in a selection of new TB drug target candidates. A number of TB drug candidates are discussed along with advancing technologies for high-throughput screening for inhibitors of latency and the use of knock-down mutants to validate targets.

Dopazo J. (2014) Genomics and transcriptomics in drug discovery. Drug Discov Today 19: 126-132

Bedard P. L., Hansen A. R., Ratain M. J. and Siu L. L. (2013) Tumour heterogeneity in the clinic. Nature 501: 355-364

Dancey J. E., Bedard P. L., Onetto N. and Hudson T. J. (2012) The genetic basis for cancer treatment decisions. Cell 148: 409-420

Pickl M., Ruge E. and Venturi M. (2012) Predictive markers in early research and companion diagnostic developments in oncology. N Biotechnol 29: 651-655

Woollard P. M., Mehta N. A., Vamathevan J. J., Van Horn S., Bonde B. K., et al. (2011) The application of next-generation sequencing technologies to drug discovery and development. Drug Discov Today 16: 512-519

Zatloukal K. and Hainaut P. (2010) Human tissue biobanks as instruments for drug discovery and development: impact on personalized medicine. Biomark Med 4: 895-903

References

Anders L., Guenther M. G., Qi J., Fan Z. P., Marineau J. J., et al. (2014) Genome-wide localization of small molecules. Nat Biotechnol 32: 92-96

A vast number of small-molecule ligands, including therapeutic drugs under development and in clinical use, elicit their effects by binding specific proteins associated with the genome. This study presents a novel method (Chem-Seq) for mapping the direct interactions of a chemical entity with chromatin genome-wide using Illumina HiSeq massively parallel DNA sequencing. The authors show how Chem-Seq can be combined with ChIP-Seq to gain unique insights into the interaction of drugs with their target proteins throughout the genome of tumor cells.

Illumina Technology: HiSeq

Braun J., Misiak D., Busch B., Krohn K. and Huttelmaier S. (2014) Rapid identification of regulatory microRNAs by miTRAP (miRNA trapping by RNA in vitro affinity purification). Nucleic Acids Res 42: e66

miRNAs are evolutionarily conserved small noncoding RNAs that regulate gene expression at the post-transcriptional level. The identification of miRNAs regulating the fate of a specific messenger RNA is a challenge due to the imperfect complementarity of miRNAs and target transcripts. This study presents the miTRAP (miRNA trapping by RNA in vitro affinity purification) protocol for miRNA identification. The authors demonstrated miTRAP for rapid identification of miRNAs using the Illumina NGS platform as a cost-effective method that allowed identification of novel miRNAs.

Illumina Technology: HiScanSQ

Jour G., Scarborough J. D., Jones R. L., Loggers E., Pollack S. M., et al. (2014) Molecular profiling of soft tissue sarcomas using next-generation sequencing: a pilot study toward precision therapeutics. Hum Pathol 45: 1563-1571

Soft tissue sarcomas (STS) are a heterogeneous group of tumors. Advances in NGS technology have prompted the incorporation of molecular data into the classification of these tumors. In this study, the authors examined the application of a targeted assay using Illumina HiSeq 2500 as a comprehensive test for mutational analysis in STS. The authors identified targetable mutations for which clinical trials were available in 60% of the cases. In addition, the analysis characterized frequent variants in STS that are currently nontargetable for existing therapies, but may serve as prognostic markers for classifying STS tumors.

Illumina Technology: HiSeq 2500

Mack S. C., Witt H., Piro R. M., Gu L., Zuyderduyn S., et al. (2014) Epigenomic alterations define lethal CIMP-positive ependymomas of infancy. Nature 506: 445-450

Ependymomas are common childhood brain tumors that occur throughout the nervous system but are most common in the hindbrain. This cancer type is characterized by not being susceptible to cytotoxic chemotherapy, and genomic analysis reveals a cancer genome almost devoid of somatic mutations. In this study, the authors investigated the methylation status of ependymomas using Illumina Infinium methylation arrays and found that ependymomas exhibit a CpG island methylation phenotype. The results suggest promoter CpG hypermethylation is involved in driving the pathogenesis of posterior fossa group A–CpG island methylator (PFA-CIMP) ependymomas and could constitute a potential new target for therapy.

Illumina Technology: HiSeq 2000, HumanMethylation450 (Infinium-GT)

Beronja S., Janki P., Heller E., Lien W. H., Keyes B. E., et al. (2013) RNAi screens in mice identify physiological regulators of oncogenic growth. Nature 501: 185-190

RNAi screening has been used successfully in cell-line assays to identify genes involved in oncogenic growth control. This paper presents the first genome-wide RNAi-mediated screens in utero for mouse embryos. Focusing on skin development and oncogenic hyperplasia, the screening uncovered previously unknown—as well as anticipated—regulators of embryonic epidermal growth.

Illumina Technology: HiSeq 2000

Pethe K., Bifani P., Jang J., Kang S., Park S., et al. (2013) Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. Nat Med 19: 1157-1160

New therapeutic strategies are needed to combat the TB pandemic and the spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) forms of the disease, which remain a serious public health challenge worldwide. This study reports on a class of imidazopyridine amide (IPA) compounds that block M. tuberculosis growth by targeting the cytochrome bc1 complex. The effect of the drug Q203 was shown first in clinical isolates in culture broth medium and was also shown to be very effective in a mouse model of tuberculosis even at a low dosage per body weight.

Illumina Technology: Genome Analyzer I

Van Vlierberghe P., Ambesi-Impiombato A., De Keersmaecker K., Hadler M., Paietta E., et al. (2013) Prognostic relevance of integrated genetic profiling in adult T-cell acute lymphoblastic leukemia. Blood 122: 74-82

Adult T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic tumor associated with poor outcome. In this study, the authors utilized Illumina BeadChip expression arrays to evaluate the prognostic value of gene expression signatures for T-ALL outcome. The authors identified an early immature gene expression signature associated with poor prognosis, and a signature of specific surface marker expression and mutations associated with improved overall survival.

Illumina Technology: Human BeadChip Array

Wu Y. M., Su F., Kalyana-Sundaram S., Khazanov N., Ateeq B., et al. (2013) Identification of targetable FGFR gene fusions in diverse cancers. Cancer Discov 3: 636-647

Recurrent gene fusions are an important class of driver mutations in cancer. Previous studies have shown *FGFR* fusions in approximately 3% of glioblastoma multiforme tumors, and *FGFR3-TACC3* fusions were identified in a subset of bladder cancers. This study examined patients with different types of cancer, including cholangiocarcinoma, breast cancer, and prostate cancer, for *FGFR* fusion genes, using Illumina HiSeq 2000 for RNA and DNA sequencing. The authors identified four cases of *FGFR2* gene rearrangements and characterized the susceptibility of fusion gene cell lines to pharmacologic inhibition in vitro and *in vivo*.

Illumina Technology: HiSeq 2000

Target Validation

Target validation refers to the stage in drug development where an identified target is validated in clinically relevant models, such as human cell lines, animal models, or primary patient tissue samples. Such models may include drug-resistant mutants of the presumed target, knock-down, or overexpression of the target. The process of validation aims at registering the direct and indirect response of the model system(s) to the drug treatment. In broad interpretation, target validation by means of NGS can be used for testing the variation between individuals for understanding disease susceptibility,[3] and for identifying the differences in biomarker levels, such as miRNA, in case vs. control samples *in vitro* and *in vivo*. To provide meaningful and interpretable information, NGS assays should: 1) measure what they claim to measure; 2) provide reproducible data; and 3) produce results that are statistically meaningful.

Over 90% of the drug candidates entering clinical trials do not demonstrate safety and efficiency sufficient to gain regulatory approval. This high degree of failure can be largely attributed to the limitations of model systems used at the target validation and pre-clinical stages of the development process[27]. Strictly speaking, target validation spans a significant portion of the drug development process, from target identification to clinical trials. The ideal "model" for validating a target is a human with naturally occurring genetic aberrations that modulate a hypothetical target with a reproducible effect on human physiology.[27] However, the power of human genetics and genetic analysis now allows the rapid generation of various human disease models in *vitro* for validating drug targets, and explaining the drug's mechanism of action, toxicity, and resistance.

One prominent new approach called "gene editing" has recently been introduced to reconstruct human genetic alterations in vitro. This approach couples the power of NGS for identification of potential target genes and drug resistance genes with a recently discovered gene editing tool for studying mutations in clustered regularly interspaced short palindromic repeats (CRISPR) and Cas-9 genes. The gene editing tool can be used both for knock-in and knock-out of genes of interest, allowing generation of heterozygous and homozygous mutant models.[28] This method can be used both for validating the gene or protein target and for unraveling genetic and epigenetic mechanisms of drug resistance in dominant and recessive drug-resistant alleles.[28 29] *(See Drug Resistance.)*

NGS also facilitates the development and verification of genetically engineered mouse models (GEMMs),[30 2] which often provide great help for target validation. This approach is replacing lengthy and costly genetic mapping studies.[2 31].

Reviews

Plenge R. M., Scolnick E. M. and Altshuler D. (2013) Validating therapeutic targets through human genetics. Nat Rev Drug Discov 12: 581-594

Woollard P. M., Mehta N. A., Vamathevan J. J., Van Horn S., Bonde B. K., et al. (2011) The application of next-generation sequencing technologies to drug discovery and development. Drug Discov Today 16: 512-519

References

Huijbers I. J., Bin Ali R., Pritchard C., Cozijnsen M., Kwon M. C., et al. (2014) Rapid target gene validation in complex cancer mouse models using re-derived embryonic stem cells. EMBO Mol Med 6: 212-225

Human cancers modeled in GEMMs can provide important mechanistic insights into the molecular basis of tumor development. This study demonstrates how 3 GEMMs (2 lung cancer models and 1 mesothelioma model) were used for fast generation of tumor cohorts. The tumor DNA was sequenced on Illumina HiSeq 2000 for copy-number analysis. This GEMM-embryonic stem cell (ESC) approach speeds up the generation/modification of mouse models, while minimizing breeding efforts.

Illumina Technology: HiSeq 2000 (Sequencing)

Kasap C., Elemento O. and Kapoor T. M. (2014) DrugTargetSeqR: a genomics- and CRISPR-Cas9-based method to analyze drug targets. Nat Chem Biol 10: 626-628

This study introduces an assay, DrugTargetSeqR, to identify physiological targets of drugs. The assay combines Illumina RNA-Seq, computational mutation discovery, and CRISPR-Cas9–based genome editing. Genetically heterogenous cancer cells are treated with the drug of interest, and resistant clones are isolated and processed for RNA-Seq CRISPR-Cas9–based genome editing. Drug-based selection is used to determine which mutations are sufficient to confer resistance, followed by biochemical validation of the drug's direct target.

Illumina Technology: HiSeq 2500

Smurnyy Y., Cai M., Wu H., McWhinnie E., Tallarico J. A., et al. (2014) DNA sequencing and CRISPR-Cas9 gene editing for target validation in mammalian cells. Nat Chem Biol 10: 623-625

Identification and validation of drug-resistant mutations can provide important insights into the mechanism of action of a compound. This paper presents a method for using gene-editing (CRISPR-Cas9) in combination with Illumina (HiSeq and MiSeq) massively parallel sequencing, for identification of drug-resistant mutations. The authors demonstrate their assay on 2 drug-resistant clones and show that disrupting the functional allele or introducing point mutations by gene editing can confer drug resistance.

Illumina Technology: HiSeq 2000, MiSeq

Wan X., Corn P. G., Yang J., Palanisamy N., Starbuck M. W., et al. (2014) Prostate cancer cell-stromal cell crosstalk via FGFR1 mediates antitumor activity of dovitinib in bone metastases. Sci Transl Med 6: 252ra122

Bone is the most common site of prostate cancer progression to a therapy-resistant, lethal phenotype. This study examined the response to the receptor tyrosine kinase inhibitor dovitinib among male patients with castration-resistant prostate cancer and bone metastases. The authors used Illumina Genome Analyzer and Illumina HiSeq 2000 for both exome sequencing and RNA-Seq of 10 cell lines, 19 noncancerous prostate cancer–adjacent tissue samples, and 136 human prostate cancer tissue samples. The authors identified a positive feedback loop between prostate cancer cells and bone cells. Their findings suggest that targeting FGFR has therapeutic activity in advanced prostate cancer.

IIllumina Technology: HiSeq 2000, Genome Analyzer

Matzuk M. M., McKeown M. R., Filippakopoulos P., Li Q., Ma L., et al. (2012) Small-molecule inhibition of BRDT for male contraception. Cell 150: 673-684

Roberts N. J., Vogelstein J. T., Parmigiani G., Kinzler K. W., Vogelstein B., et al. (2012) The predictive capacity of personal genome sequencing. Sci Transl Med 4: 133ra158

Sedaghat Y., Mazur C., Sabripour M., Hung G. and Monia B. P. (2012) Genomic analysis of wig-1 pathways. PLoS One 7: e29429

Biomarkers

New demands from health plans and health care providers to improve the quality of health care without increasing overall costs will increase the demand for methods that demonstrate improvements in health outcomes over existing treatments. Biomarkers are the signature molecules used for the identification of patients who can benefit from treatment with a specific drug.[32] Biomarkers are used as diagnostic targets in companion diagnostic tests.[32] While the number of new drugs with associated diagnostics has followed linear growth patterns, the use of advanced diagnostics for therapy selection has shown exponential growth. The identification of biomarkers for safety, sensitivity, and resistance for on-market drugs further drive value in research efforts and clinical needs.

The identification and use of biomarkers for diagnostic and therapeutic purposes has been increasingly valued by regulatory agencies such as the US Food and Drug Administration (FDA), which has developed a Critical Path Initiative (CPI). The CPI is the "FDA's national strategy for transforming the way FDA-regulated medical products are developed, evaluated, and manufactured."[33] This program aims at facilitating the development of innovative scientific tools for drug development, evaluation, and manufacturing.[7] In a separate document, the FDA strongly recommends pharmaceutical companies to develop new therapeutic agents in conjunction with in vitro diagnostic (IVD) companion diagnostics. It warns that new drugs deemed by the FDA to require such companion diagnostics will not be approved until both components are tested and submitted for the agency's approval.[34] Common applications of biomarkers are shown in Table 2.

Table 2. Application of biomarkers in drug development[35]

Type of biomarker	Questions answered	Information provided
Predictive	Which drug(s)?	Response to targeted treatment
Prognostic	Who needs treatment?	Clinical outcome
Pharmacogenomic	What dose is needed?	Drug metabolism

In 2001, the Early Detection Research Network of the National Cancer Institute (NCI) established a formal process to guide biomarker development for early disease detection.[36] Five phases of this process are summarized in Table 3. The process was originally designed for cancer-specific biomarkers but eventually was extended to other disease categories.

Table 3: Phases of Biomarker Development [37]

Phase	Type of Experiment	Goal
1	Preclinical exploratory	Identify promising biomarkers
2	Clinical assay and validation	Develop clinical assay to distinguish diseased from nondiseased or responders from nonresponders
3	Retrospective longitudinal	Verify if the biomarker can detect the outcome of interest
4	Prospective study	Test if the biomarker can do what it is supposed to do, and determine the positive predictive value
5	(Cancer) control	Quantify how the biomarker performs in a population (determine clinical validity)

From a payer perspective, the number of nonresponders in some therapeutic areas, such as rheumatoid arthritis, is greater than 50%. Significant value can be obtained by applying markers that predict response and reduce the waste of drugs on nonresponders while subjecting those patients to unnecessary drug-associated adverse events.

Reviews

Miller F. A., Hayeems R. Z., Bytautas J. P., Bedard P. L., Ernst S., et al. (2014) Testing personalized medicine: patient and physician expectations of next-generation genomic sequencing in late-stage cancer care. Eur J Hum Genet 22: 391-395

Olsen D. and Jorgensen J. T. (2014) Companion diagnostics for targeted cancer drugs - clinical and regulatory aspects. Front Oncol 4: 105

Zieba A., Grannas K., Soderberg O., Gullberg M., Nilsson M., et al. (2012) Molecular tools for companion diagnostics. N Biotechnol 29: 634-640

Zatloukal K. and Hainaut P. (2010) Human tissue biobanks as instruments for drug discovery and development: impact on personalized medicine. Biomark Med 4: 895-903

References

Ong M., Carreira S., Goodall J., Mateo J., Figueiredo I., et al. (2014) Validation and utilisation of high-coverage next-generation sequencing to deliver the pharmacological audit trail. Br J Cancer 111: 828-836

Predictive biomarker development is a key challenge for novel cancer therapeutics. This study explored the feasibility of NGS to validate genomic biomarkers that impact phase I trial selection. The study used the Illumina MiSeq TruSeq Amplicon Cancer Panel (TSACP) for targeted tumor sequencing and Illumina MiSeq validation in a separate cohort. The authors concluded that targeted high-coverage NGS panels are a highly feasible technology to enrich trials with molecularly defined populations and support hypothesis testing early in drug development.

Illumina Technology: MiSeq

Thong A. E., Zhao H., Ingels A., Valta M. P., Nolley R., et al. (2014) Tissue slice grafts of human renal cell carcinoma: an authentic preclinical model with high engraftment rate and metastatic potential. Urol Oncol 32: 43 e23-30

Tumor grafts, the direct implantation of patient-derived tissues into mice, have demonstrated their value in drug validation and evaluation of therapeutic response. In this study, the authors evaluate tissue-slice grafts (TSGs) as an improved tumor graft model of renal cell carcinoma. The TSGs were evaluated by RNA expression profiling on Illumina BeadChip arrays and direct DNA sequencing. The authors found that their TSGs captured the pathology, gene expression, genetic mutation, and drug response of tumors and recommend TSGs as a preclinical model.

Illumina Technology: BeadChip array

Preclinical Development

Metabolism and Toxicity of Drugs and Vaccines

Toxicology studies based on the use of high-throughput techniques ("omics") that employ bioinformatics tools are termed "toxicogenomics." The "omics" techniques include transcriptomics, epigenomics, global miRNA expression, proteomics, and metabolomics.[7]

The association between genotypes and hypertension was examined in breast cancer patients treated with bevacizumab, a broad anticancer therapy, in a phase III trial. A SNV in the synaptic vesicle glycoprotein (SV2C) gene was determined by genome-wide association studies (GWAS) and validated in another randomized phase III trial. This approach allowed the identification of a predictive biomarker for bevacizumab-induced hypertension.[38]

Breast cancer patients treated with cyclophosphamide and doxorubicin often develop hematological and gastrointestinal toxicities. Yao et al. used the Illumina GoldenGate platform to determine that these toxicities are related to 6 single-nucleotide polymorphisms (SNPs) in pharmacokinetic genes, as demonstrated in a phase III Southwest Oncology Group (SWOG) clinical trial.[39 40] This diagnostic assay can help assess the risk of toxic reactions before prescribing chemotherapy.[39]

Ivanov et al. have developed a modified method for targeted bisulfite sequencing of 174 absorption, distribution, metabolism, and excretion (ADME) genes.[41] The method combines standard in-solution hybrid capture with DNA bisulfite treatment.

Vaccine Development (Including Tumor Vaccination)

Viruses exist as heterogeneous and complex populations comprising similar but not identical genomes. NGS can be used to characterize the population, including rare members, with a very high degree of accuracy[42]. The immune response of the host can also be measured, as well as the T-cell response and memory.[43 44] NGS was also used in identification of the new strain of the Ebola virus where quantitative polymerase chain reaction (qPCR) showed negative results.[45] A deeper understanding of the host-pathogen response promises to greatly improve the speed and success of vaccine development.

NGS also can be used for vaccine quality control. Rentsch et al. have recently utilized this method to plot correlation between mutations accumulated in 2 major laboratory-cultivated passages derived from the original 1921 strain of intravesical instillation of bacillus Calmette-Guérin (BCG) and clinical efficiency of this vaccine in treatment of patients with non–muscle-invasive bladder cancer (NMIBC).[46]

Reviews

Khan S. R., Baghdasarian A., Fahlman R. P., Michail K. and Siraki A. G. (2014) Current status and future prospects of toxicogenomics in drug discovery. Drug Discov Today 19: 562-578

References

Li X., Qu F., Xie W., Wang F., Liu H., et al. (2014) Transcriptomic analyses of neurotoxic effects in mouse brain after intermittent neonatal administration of thimerosal. Toxicol Sci 139: 452-465

Thimerosal is an antimicrobial preservative in vaccines that has been associated with adverse neurotoxic effects, including autism. This study leverages high-throughput RNA sequencing of autistic-behaved mouse brains to reveal alteration of a number of canonical pathways involving neuronal development, synaptic function, and endocrine dysregulation. High-throughput RNA sequencing enabled the researchers to obtain a comprehensive transcriptomic comparison between normal and thimerosal-treated autistic-like mouse brains. Subsequent pathway analyses revealed that neuronal axon guidance is most affected. Dysregulation of neurodevelopment has been implicated in neurologic sequelae and behavior abnormalities.

Illumina Technology: HiSeq 2000

Schneider B. P., Li L., Shen F., Miller K. D., Radovich M., et al. (2014) Genetic variant predicts bevacizumab-induced hypertension in ECOG-5103 and ECOG-2100. Br J Cancer 111: 1241-1248

Bevacizumab is a monoclonal antibody that targets vascular endothelial growth factor A (VEGFA). Bevacizumab has broad antitumor activity but substantial risk of hypertension. This study presents a GWAS in a phase III bevacizumab-based adjuvant breast cancer trial and a second trial for metastatic disease to evaluate an association between genotypes and hypertension. Genotyping was performed using Illumina BeadChip (Infinium GT) microarrays for a total of 4,994 patients. The study identified a genetic variant in SV2C that predicted clinically relevant bevacizumab-induced hypertension in both randomized phase III trials.

Illumina Technology: HumanOmni1-Quad (Infinium GT), HumanOmniExpress (Infinium GT)

Thong A. E., Zhao H., Ingels A., Valta M. P., Nolley R., et al. (2014) Tissue slice grafts of human renal cell carcinoma: an authentic preclinical model with high engraftment rate and metastatic potential. Urol Oncol 32: 43 e23-30

Tumor grafts, the direct implantation of patient-derived tissues into mice, have demonstrated their value in drug validation and evaluation of therapeutic response. In this study, the authors evaluate TSGs as an improved tumor graft model of renal cell carcinoma (RCC). The TSGs were evaluated by RNA expression profiling on Illumina BeadChip arrays and direct DNA sequencing. The authors found that TSGs capture the pathology, gene expression, genetic mutation, and drug response of tumors. They recommend the use of TSGs as a preclinical model.

Illumina Technology: Infinium BeadChip Array

Yao S., Sucheston L. E., Zhao H., Barlow W. E., Zirpoli G., et al. (2014) Germline genetic variants in ABCB1, ABCC1 and ALDH1A1, and risk of hematological and gastrointestinal toxicities in a SWOG Phase III trial S0221 for breast cancer. Pharmacogenomics J 14: 241-247

Hematological and gastrointestinal toxicities are common among patients treated with cyclophosphamide and doxorubicin for breast cancer. In this study, the authors used Illumina GoldenGate arrays to study the genotypes ABCB1, ABCC1, and ALDH1A1 in 882 breast cancer patients in relation to risk of high-grade toxicities after treatment with doxorubicin and cyclophosphamide. None of the ABCB1 SNPs was associated with either toxicity, but 3 SNPs in ABCC1 and 3 SNPs and one haplotype in ALDH1A1 were associated with grade 3 and 4 hematological toxicity.

Illumina Technology: GoldenGate array

Buchovecky C. M., Turley S. D., Brown H. M., Kyle S. M., McDonald J. G., et al. (2013) A suppressor screen in Mecp2 mutant mice implicates cholesterol metabolism in Rett syndrome. Nat Genet 45: 1013-1020

Mutations in the gene encoding methyl CpG-binding protein 2 (MECP2) cause Rett syndrome, the most severe autism-spectrum disorder. Previous work showed that re-expressing Mecp2 in symptomatic Mecp2-null mice markedly improves function and longevity, providing hope that therapeutic intervention is possible in humans. This study examined pathways in disease pathology for therapeutic intervention by studying a mouse model of Rett syndrome (Mecp2-null mice) with an Illumina SNP panel and exome sequencing. The genetic screen suggests that cholesterol homeostasis is a potential target for the treatment of Rett syndrome.

Illumina Technology: HiSeq 2000, Genome Analyzer

Zhang, J., Benavente C. A., McEvoy J., Flores-Otero J., Ding L., et al. (2012) A novel retinoblastoma therapy from genomic and epigenetic analyses. Nature 481: 329-334

Retinoblastoma is an aggressive childhood cancer of the developing retina that is initiated by RB1 inactivation, but the underlying mechanism is not known. In a highly aggressive cancer such as this, many genes are involved but RB1 was the only known cancer gene mutated. In contrast to the limited number of somatic mutations, the tumor showed profound changes in its methylation profile relative to in normal retinoblasts. One of the most striking results was the induction of the expression of the proto-oncogene spleen tyrosine kinase (SYK) gene in human retinoblastoma. SYK is required for tumor cell survival. The researchers went on to show that inhibition of SYK expression with a small-molecule inhibitor caused cell death in retinoblastoma cells in culture and *in vivo*.

Illumina Technology: Methylation 27 arrays, Genome Analyzer$_{IIx}$ 101-bp paired-end both targeted and whole genome

Ivanov M., Kals M., Kacevska M., Metspalu A., Ingelman-Sundberg M., et al. (2013) In-solution hybrid capture of bisulfite-converted DNA for targeted bisulfite sequencing of 174 ADME genes. Nucleic Acids Res 41: e72

The coupling of target enrichment techniques with bisulfite conversion of DNA enabled researchers to investigate gene expression within ADME pathways. The methylation levels of 41,922 CpG sites in target regions were studied with both sequencing and BeadChip arrays to reveal 1,702 sites of differential methylation among 4 human liver samples. The authors conclude that genomic regions of intermediate CpG density (CGI shores) merit greater focus when studying interindividual variation in human liver DNA methylation and that such studies will further contribute to understanding epigenomic influences on drug ADME.

Illumina Technology: HiSeq 2000, HumanMethylation450 BeadChip Array

Matthews G. M., Lefebure M., Doyle M. A., Shortt J., Ellul J., et al. (2013) Preclinical screening of histone deacetylase inhibitors combined with ABT-737, rhTRAIL/MD5-1 or 5-azacytidine using syngeneic Vk*MYC multiple myeloma. Cell Death Dis 4: e798

Many patients with multiple myeloma (MM) fail to respond to therapy, or relapse after an initial response. This study investigated the efficacy and safety of histone deacetylase inhibitors (HDACi) and combination therapies in vitro in human MM cell lines. The authors used Illumina HiSeq for RNA-Seq to monitor the expression response to HDACi treatment. They found that, although HDACi and combination therapies demonstrated an effect, they also showed on-target dose-limiting toxicities that preclude prolonged treatment.

Illumina Technology: HiSeq

Clinical Development

Patient Stratification and Drug Rescue

Historically, the interest of pharmaceutical companies in tracking and studying statistical outliers in clinical trials was low. Both exceptional responders and nonresponders were negatively affecting the outcomes of traditional randomized trials. Recently, exceptional responders—patients with unusually strong positive reaction to drugs—have started attracting significant attention from clinical researchers.[47] Identification of genomic drivers of this unusual response may guide modification of existing drugs, the development of companion diagnostics and combination therapies, and/or stratification of patients in new clinical trials and retrospectively. Exceptional responders with various types of cancer will be studied in 2 large scale projects in the Memorial Sloan-Kettering Cancer Center (MSKCC) in New York and the NCI. The NCI project aims at performing a thorough genomic analysis of 100 oncology patients that have exhibited pronounced responses to therapy in clinical trials that would otherwise be deemed a failure. Other major academic cancer centers, including the Dana-Farber Cancer Institute, Massachusetts General Hospital, the Broad Institute, and the MD Anderson Cancer Center have also started collecting and systemizing information on exceptional responders across various trials.[47]

Historically, the first WGS study of an exceptional (profound) responder was conducted in the MSKCC in a patient with advanced bladder cancer. This patient demonstrated a durable (>2 years in remission) and ongoing complete response to the mammalian target of rapamycin complex (mTORC1) inhibitor Afinitor (everolimus) in the phase II clinical trial.[48,49] Sequencing this patient's genome allowed the identification of a loss-of-function mutation in the tuberous sclerosis complex 1 (TSC1) gene, which can open up a new treatment opportunity for 6–8% of bladder cancer patients, as well as those with subendymal giant cell astrocyma, who frequently possess the same mutation.[48] Other prominent examples of extreme responders identified using NGS are a patient with metastatic small cell cancer[50] treated with irinotecan (WGS approach) and another patient with bladder cancer treated with Afinitor (WES approach).[51]

The MSKCC has recently developed an Integrated Mutational Profiling of Actionable Cancer Targets (IMPACT) assay, based on hybrid capture followed by sequencing on the Illumina HiSeq platform. This assay will sequence 341 cancer-associated genes from each sample.[47] Another similar system, FoundationOne, was codeveloped by the MSKCC and Foundation Medicine, a cancer genomics analysis company, and will be used to screen for more than 400 genes in patients with hematologic malignancies.[47] The validation study for this platform was published in *Nature Biotechnology* in 2013.[52]

"They found some mutations, which are untypical for my kind of cancer, but are very well studied for other cancers. And therefore there are actually three options."

Anonymous patient with advanced solid tumor, Miller et al. 2014

Sheridan et al. have highlighted a need to build an online registry of mutations in extreme responders and nonresponders, to predict treatment outcomes in larger patient populations.[47] As one of the major undertakings of this idea, the NCI has recently established an NCI Molecular Analysis for Therapy Choice Program (NCI-MATCH) initiative, a large-scale trial to test multiple therapeutic agents in patients with various types of cancer, selected based on their genetic profile.[47 53] This trial will begin in 2015 and is expected to recruit up to 3,000 individuals.

Stratification of patients in clinical trials and running trials retrospectively is often based on the analysis of archived biopsy samples. Primary samples are often only available in a form of formalin-fixed, paraffin embedded (FFPE) samples. The FFPE fixation procedure may damage DNA.[54] Modified NGS library preparation protocols and computational methods have recently been established for running WES on such samples.[52 55] Frampton et al. have developed a diagnostic test for detection of alterations in 4,557 exons of 287 cancer-related genes based on the Illumina HiSeq platform. The authors have reported 95–99% test and high sensitivity (positive predictive value >99%). The test allowed detection of actionable alterations in 76% of tumors, reportedly 3 times the number of such alterations detected by currently used diagnostic tests, such as PCR, Sanger sequencing, mass spectrometric genotyping, immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH).[52] Another similar NGS-based test for detecting oncology alterations in FFPE samples, UW-OncoPlex, was developed by Pritchard et al. UW-OncoPlex is a clinical molecular diagnostic assay aimed at providing simultaneous deep-sequencing information for all classes of mutations in 194 clinically relevant cancer genes.[56] The studies of Pritchard et al. and Jour et al. demonstrate that NGS assays are already capable of providing valuable predictive and prognostic information from clinical samples, and they can have an immediate impact on patient care.[56 57]

Reviews

Sheridan C. (2014) Cancer centers zero in on exceptional responders. Nat Biotechnol 32: 703-704

Traditionally, anecdotal case reports of exceptional responders have commanded low levels of interest from journal editors, and pharmaceutical companies have had little interest in pursuing rare outliers in drug trials. But with the increasing power and falling cost of NGS technologies, it is now possible to identify the genetic variations underpinning those exceptional responses. The MSKCC will use the IMPACT system with Illumina HiSeq sequencing to analyze solid tumor samples from exceptional responders to therapy. Given the high numbers of patients that will be screened, MSKCC directors expect even rare mutations to be captured and present new drug targets for more efficient personalized therapies.

Illumina Technology: HiSeq 2500

Miller F. A., Hayeems R. Z., Bytautas J. P., Bedard P. L., Ernst S., et al. (2014) Testing personalized medicine: patient and physician expectations of next-generation genomic sequencing in late-stage cancer care. Eur J Hum Genet 22: 391-395

Wagle, N., Berger M. F., Davis M. J., Blumenstiel B., DeFelice M., et al. (2011) High-Throughput Detection of Actionable Genomic Alterations in Clinical Tumor Samples by Targeted, Massively Parallel Sequencing. Cancer Discovery 2: 82-93

Pant S., Weiner R. and Marton M. J. (2014) Navigating the rapids: the development of regulated next-generation sequencing-based clinical trial assays and companion diagnostics. Front Oncol 4: 78

Bedard P. L., Hansen A. R., Ratain M. J. and Siu L. L. (2013) Tumour heterogeneity in the clinic. Nature 501: 355-364

Simon R. and Roychowdhury S. (2013) Implementing personalized cancer genomics in clinical trials. Nat Rev Drug Discov 12: 358-369

Holbrook, J. D., Parker J. S., Gallagher K. T., Halsey W. S., Hughes A. M., et al. (2011) Deep sequencing of gastric carcinoma reveals somatic mutations relevant to personalized medicine. J Transl Med 9: 119

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Al-Ahmadie H., Iyer G., Hohl M., Asthana S., Inagaki A., et al. (2014) Synthetic Lethality in ATM-Deficient RAD50-Mutant Tumors Underlies Outlier Response to Cancer Therapy. Cancer Discov 4: 1014-1021

Metastatic solid tumors are almost invariably fatal. In this report, the authors present a case study of an exceptional responder who achieved a complete response to therapy. Using WGS on Illumina HiSeq, the authors identified a clonal homozygous mutation in the RAD50 gene that contributed (via synthetic lethality) to extreme sensitivity to irinotecan treatment. The authors suggest targeted tumor-specific combination therapy, where checkpoint inhibition in combination with DNA-damaging chemotherapy will prove synthetically lethal in tumor cells.

Illumina Technology: HiSeq 2000

Choi W., Porten S., Kim S., Willis D., Plimack E. R., et al. (2014) Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. Cancer Cell 25: 152-165

Muscle-invasive bladder cancers (MIBCs) are biologically heterogeneous and have widely variable clinical outcomes and responses to conventional chemotherapy. In this study of MIBC expression, the authors used Illumina expression arrays to discover 3 molecular subtypes of MIBC: 1) basal MIBCs; 2) luminal MIBCs; and 3) p53-like MIBCs that adopted a p53-like phenotype after therapy. The characterization of these tumor subtypes will have important implications for improving the prognosis and development of targeted therapies.

Illumina Technology: Human BeadChip array, DASL (Gene Expression - BeadArray)

Gottardo N. G., Hansford J. R., McGlade J. P., Alvaro F., Ashley D. M., et al. (2014) Medulloblastoma Down Under 2013: a report from the third annual meeting of the International Medulloblastoma Working Group. Acta Neuropathol 127: 189-201

Medulloblastoma is curable in approximately 70 % of patients. However, over the past decade, progress in Medulloblastoma is curable in approximately 70% of patients. However, over the past decade, progress in improving survival using conventional therapies has stalled. This report describes the Medulloblastoma Down Under 2013 meeting, which convened at Bunker Bay, Australia. The meeting brought together 50 leading clinicians and scientists in focused sessions on the latest development in pathology and molecular stratification, genomics and mouse models, high-throughput drug screening, and clinical trial design. Several presenters described how they used Illumina technology in their experimental design for expression and epigenetics profiling.

Illumina Technology: HumanMethylation450 BeadChip Array

Johnson D. B., Dahlman K. H., Knol J., Gilbert J., Puzanov I., et al. (2014) Enabling a genetically informed approach to cancer medicine: a retrospective evaluation of the impact of comprehensive tumor profiling using a targeted next-generation sequencing panel. Oncologist 19: 616-622

NGS is a powerful tool to identify tumor-specific genetic changes. This study performed a retrospective assessment of the NGS results and therapies received for patients undergoing targeted NGS for selected cancer genes using Illumina HiSeq for sequencing. The authors concluded that mutational profiling using a targeted NGS panel successfully identified potentially actionable genetic variants in the majority of patients. The assay additionally identified alternative therapeutic options and facilitated clinical trial enrollment.

Illumina Technology: HiSeq 2000

Le Morvan V., Litiere S., Laroche-Clary A., Ait-Ouferoukh S., Bellott R., et al. (2014) Identification of SNPs associated with response of breast cancer patients to neoadjuvant chemotherapy in the EORTC-10994 randomized phase III trial. Pharmacogenomics J

The efficacy of anticancer drugs is highly variable among individuals. This GWAS analyzed genotypes and clinical outcomes for breast cancer patients. The authors used Illumina GoldenGate arrays to genotype 384 selected SNPs in the germline DNA extracted from FFPE noninvaded lymph nodes of 243 patients. Three polymorphisms were found to significantly associate with pathological complete response in patients harboring a p53-positive tumor.

Illumina Technology: Custom GoldenGate Array

Ong M., Carreira S., Goodall J., Mateo J., Figueiredo I., et al. (2014) Validation and utilisation of high-coverage next-generation sequencing to deliver the pharmacological audit trail. Br J Cancer 111: 828-836

Predictive biomarker development is a key challenge for novel cancer therapeutics. This study explored the feasibility of using NGS to validate genomic biomarkers that impact phase I trial selection. Using the Illumina MiSeq TSACP for targeted tumor sequencing and Illumina MiSeq validation in a separate cohort, the authors concluded that targeted high-coverage NGS panels are a highly feasible technology to enrich trials with molecularly defined populations and support hypothesis testing early in drug development.

Illumina Technology: MiSeq

Tobiasson M., Dybedahl I., Holm M. S., Karimi M., Brandefors L., et al. (2014) Limited clinical efficacy of azacitidine in transfusion-dependent, growth factor-resistant, low- and Int-1-risk MDS: Results from the nordic NMDSG08A phase II trial. Blood Cancer J 4: e189

The myelodysplastic syndromes (MDSs) constitute a group of malignant hematopoietic stem cell disorders that carry a significant risk for progression to acute myeloid leukemia. In this phase II trial, the authors tested the efficacy and response of azacitidine + erythropoietin in transfusion-dependent patients with lower-risk MDS. All patients were analyzed for 42 genes recurrently mutated in myeloid disorders using targeted sequencing on the Illumina HiSeq 2000 system. The screening revealed a high frequency of recurrent mutations. Although no single mutation predicted for response, 2 mutations were observed only in nonresponders.

Illumina Technology: HiSeq 2000

Van Allen E. M., Wagle N., Stojanov P., Perrin D. L., Cibulskis K., et al. (2014) Whole-exome sequencing and clinical interpretation of formalin-fixed, paraffin-embedded tumor samples to guide precision cancer medicine. Nat Med 20: 682-688

The clinical use of WES and similar NGS protocols requires methods for robust and rapid extraction of DNA from FFPE tumor tissue. This study describes a prospective clinical WES platform for archival FFPE tumor samples. The authors used an optimized protocol for DNA extraction followed by sequencing on Illumina HiSeq. When applied retrospectively to 511 exomes, the interpretative framework revealed a “long tail” of somatic alterations in clinically important genes. Prospective application of this approach identified clinically relevant alterations in 15 out of 16 patients.

Illumina Technology: HiSeq 2000

Wagle N., Grabiner B. C., Van Allen E. M., Hodis E., Jacobus S., et al. (2014) Activating mTOR mutations in a patient with an extraordinary response on a phase I trial of everolimus and pazopanib. Cancer Discov 4: 546-553

Exceptional responders to cancer treatment may provide clues to understanding the genetic mechanisms of disease and treatment response. The authors present a case study of an exceptional responder in a phase 1 study of pazopanib and everolimus. Using Illumina HiSeq WES, the authors identified 2 concurrent mutations in the MTOR gene, the target of everolimus. Follow-up in vitro experiments demonstrated that both mutations were activating, suggesting a biological mechanism for everolimus sensitivity in this patient.

Illumina Technology: HiSeq 2000

Wagle N., Grabiner B. C., Van Allen E. M., Amin-Mansour A., Taylor-Weiner A., et al. (2014) Response and acquired resistance to everolimus in anaplastic thyroid cancer. N Engl J Med 371: 1426-1433

Therapies targeted at tumors with specific genetics may only be effective for a limited period until the tumor develops resistance to the treatment. Everolimus, an inhibitor of mTOR, is effective in treating tumors harboring alterations in the mTOR pathway. This case study describes a patient with metastatic thyroid carcinoma who exhibited an extraordinary 18-month response to everolimus. The authors used Illumina sequencing to characterize the tumor genome and normal blood genome before therapy, during therapy, and after acquired resistance. The resistant tumor genome revealed a mutation in the MTOR gene that confers resistance to allosteric mTOR inhibition.

Illumina Technology: HiSeq 2000

Frampton G. M., Fichtenholtz A., Otto G. A., Wang K., Downing S. R., et al. (2013) Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol 31: 1023-1031

The genetic variations specific for an individual tumor cell may determine the susceptibility of the tumor to therapies and guide prognosis. This study describes a genetic test based on massively parallel DNA sequencing to characterize base substitutions, short insertions and deletions (indels), copy number alterations, and selected fusions across 287 cancer-related genes from routine FFPE clinical specimens. The authors demonstrated the protocol using Illumina HiSeq sequencing and confirmed the accuracy in 249 FFPE cancer specimens. They applied the test to 2,221 clinical cases to reveal clinically actionable alterations in 76% of tumors.

Illumina Technology: HiSeq 2000

Drug Efficiency and Combination Drugs

A combinatory approach to the treatment of cancers has been used for over 60 years; however, identification of the drug combinations has been often based on empirical observations of the treatment outcomes. Hence, even successful drug combinations failed to cure many patients with seemingly identical malignancies.[58 59] Combination drugs can either enhance the action of one another, or have an additive effect from addressing 2 independent targets/pathways. The use of RNAi for a consecutive interruption of signaling pathways affected by combination drugs is one of the most prominent ways to identify the efficiency of combination drug therapy.[57]

Reviews

Pritchard J. R., Bruno P. M., Gilbert L. A., Capron K. L., Lauffenburger D. A., et al. (2013) Defining principles of combination drug mechanisms of action. Proc Natl Acad Sci U S A 110: E170-179

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Mithraprabhu S., Khong T. and Spencer A. (2014) Overcoming inherent resistance to histone deacetylase inhibitors in multiple myeloma cells by targeting pathways integral to the actin cytoskeleton. Cell Death Dis 5: e1134

Recent evidence has indicated that combination of HDACi and proteasome inhibitors (PI) is beneficial only in a subset of patients with advanced MM. This study examined the molecular signature associated with inherent resistance to HDACi in human myeloma cell lines (HMCL) categorized as sensitive, intermediate, or resistant to HDACi. The authors used Illumina BeadArrays to evaluate gene expression profiles. They showed that, when HMCL and primary MM samples were treated with a combination of HDACi and agents targeting the signaling pathways, integral to the actin cytoskeleton, synergistic cell death was observed in all instances.

Illumina Technology: HT-12 Infinium BeadChip Arrays

Westin J. R., Chu F., Zhang M., Fayad L. E., Kwak L. W., et al. (2014) Safety and activity of PD1 blockade by pidilizumab in combination with rituximab in patients with relapsed follicular lymphoma: a single group, open-label, phase 2 trial. Lancet Oncol 15: 69-77

In vitro studies have previously provided strong evidence that immunotherapy can induce meaningful clinical remission in follicular lymphoma. This stage 2 clinical trial evaluated the efficacy of treating follicular lymphoma patients with a combination of pidilizumab and rituximab. The authors used Illumina BeadArrays to monitor gene expression changes as a result of treatment. They concluded that the combination of pidilizumab and rituximab was active and well tolerated in patients with relapsed follicular lymphoma, with an objective response noted in 19 of 29 patients and no autoimmune or treatment-related adverse events.

Illumina Technology: HT-12 Infinium BeadChip Arrays

Matthews G. M., Lefebure M., Doyle M. A., Shortt J., Ellul J., et al. (2013) Preclinical screening of histone deacetylase inhibitors combined with ABT-737, rhTRAIL/MD5-1 or 5-azacytidine using syngeneic Vk*MYC multiple myeloma. Cell Death Dis 4: e798

Many patients with MM fail to respond to therapy, or relapse after an initial response. This study investigated the efficacy and safety of HDACi and combination therapies in vitro in human MM cell lines. The authors used Illumina HiSeq for RNA-Seq to monitor the expression response to HDACi treatment. They found that, although HDACi and combination therapies demonstrated an effect, they also showed on-target dose-limiting toxicities that preclude prolonged treatment.

Illumina Technology: HiSeq

Tabernero J., Shapiro G. I., LoRusso P. M., Cervantes A., Schwartz G. K., et al. (2013) First-in-humans trial of an RNA interference therapeutic targeting VEGF and KSP in cancer patients with liver involvement. Cancer Discov 3: 406-417

RNAi is a potent and specific mechanism for regulating gene expression. RNAi delivery mechanisms are being investigated as a potential new class of therapeutics. This study presents a trial of ALN-VSP, a lipid nanoparticle formulation of siRNAs targeting vascular endothelial growth factor (VEGF) and kinesin spindle protein (KSP) in patients with cancer. The authors monitored gene expression downregulation using Illumina RNA-Seq and showed antitumor activity, including complete regression of liver metastases in endometrial cancer. These findings show great promise for the safety, pharmacokinetics, and clinical activity for a novel first-in-class RNAi therapeutic agent in humans.

Illumina Technology: MiSeq

Companion Diagnostics

As defined by the FDA, a companion diagnostic is a device that "can be an in vitro diagnostic device or an imaging tool that provides information that is essential for the safe and effective use of a corresponding therapeutic product."[60] The definition of the "device" is broad, and both NGS and microarrays would fall under this definition. The concept of a companion diagnostic was first implemented in 1998, when HercepTest—a diagnostic tool developed by DakoCytomation to select patients for treatment with Herceptin (trastuzumab)—was approved by the FDA.[61] Translational oncology has evolved dramatically over the past 5 years, characterized by the introduction of NGS into the clinic, wherein molecular tumor classification has permitted molecular targeting (as opposed to broadly cytotoxic) anticancer therapies.

Pick et al. define a companion diagnostic as "a highly validated test that serves to qualify a patient for treatment with a particular drug or for the continued use of a particular drug." The variety of companion diagnostics available on the market is low, but this number is expected to grow rapidly as many new tests get approval following the ongoing multiple clinical trials.[32] The path from development to approval takes 5 years, on average. The clinical phase of this development process takes about 3 years and consists of 3 stages: validation, utility, and impact of the diagnostic.[61] In the development course of biomarkers, the intended purpose for method validation is entangled with the development phases of a potential drug. As pharmacodynamic, monitoring, prognostic, predictive or surrogate biomarkers have myriad intended uses, the rigor of the NGS assay will depend on the intended use of the biomarker, and will increase with each developmental phase, from discovery to validation for the intended clinical purpose.

One of the challenges in the development of companion diagnostics is the need to align the drug and diagnostic development timelines. This alignment might be challenging in cases where the biomarker for companion diagnostics is not identified until later stages in the drug development process.[62] A biomarker has to be identified as genetically stable, play a pivotal role in the disease, and be common in a relatively homogenous patient population.[32] Biomarkers are often defined based on the analysis of a large number of archived samples from biobanks. Biobanking, which is becoming a routine procedure in clinical trials, is anticipated to significantly streamline the process of companion diagnostic development.[63]

In August 2014, the US FDA developed guidelines requiring targeted drugs submitted for agency approval to be accompanied by the matching companion diagnostics.[64] Similar actions toward creating a regulatory environment friendly to the development of biomarkers and drugs for personalized medicine have been undertaken by the European Medicines Agency (EMA).[103] A full list of FDA-approved companion diagnostics is available on the FDA website[104] and in the review by Olsen at al.[66] Recent clearance of MiSeqDx by the FDA has paved the way for the adoption of NGS-based companion diagnostics in clinical and molecular diagnostics practices.[66]

Reviews

Olsen D. and Jorgensen J. T. (2014) Companion diagnostics for targeted cancer drugs - clinical and regulatory aspects. Front Oncol 4: 105

Pant S., Weiner R. and Marton M. J. (2014) Navigating the rapids: the development of regulated next-generation sequencing-based clinical trial assays and companion diagnostics. Front Oncol 4: 78

Cheng S., Koch W. H. and Wu L. (2012) Co-development of a companion diagnostic for targeted cancer therapy. N Biotechnol 29: 682-688

Pickl M., Ruge E. and Venturi M. (2012) Predictive markers in early research and companion diagnostic developments in oncology. N Biotechnol 29: 651-655

Zieba A., Grannas K., Soderberg O., Gullberg M., Nilsson M., et al. (2012) Molecular tools for companion diagnostics. N Biotechnol 29: 634-640

Zatloukal K. and Hainaut P. (2010) Human tissue biobanks as instruments for drug discovery and development: impact on personalized medicine. Biomark Med 4: 895-903

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Comprehensive molecular portraits of human breast tumours accessed at http://www.nature.com/nature/journal/v490/n7418/full/nature11412.html

Comprehensive molecular characterization of human colon and rectal cancer accessed at http://www. nature.com/nature/journal/v487/n7407/full/nature11252.html

Comprehensive genomic characterization of squamous cell lung cancers accessed at http://www.nature. com/nature/journal/v489/n7417/full/nature11404.html

Post-approval studies

Drug Re-purposing

Rapidly accumulating amount of data from WGS and WES and its systematization in databases like NextBio, Human Gene Mutation Database (HGMD), Informatics for Integrating Biology and the Bedside (i2b2), and others have identified a number of mutations common for various types of cancer and targetable via existing and developing drugs,[3 67] These findings open an opportunity to test existing drugs and drug combinations against new diseases and indications.

The idea of drug repurposing has recently advanced to a large-scale pilot project: $20 million will be spent by US funders to bring 58 abandoned drug candidates provided by 8 companies back to academic benches for finding new applications. A project of the same kind and scale was initiated in 2011 by the UK Medical Research Council, which facilitates a further study of 22 compounds previously abandoned by AstraZeneca[68]. Monitoring disease status and recurrence offers an additional application area for NGS.[3]

Reviews

Mullard A. (2012) Drug repurposing programmes get lift off. Nat Rev Drug Discov 11: 505-506

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Pritchard C. C., Salipante S. J., Koehler K., Smith C., Scroggins S., et al. (2014) Validation and implementation of targeted capture and sequencing for the detection of actionable mutation, copy number variation, and gene rearrangement in clinical cancer specimens. J Mol Diagn 16: 56-67

NGS technologies hold considerable promise for transforming clinical molecular testing of cancers, allowing comprehensive detection of actionable mutations, irrespective of cancer type. In this paper, the authors present the UW-OncoPlex pipeline: a clinical molecular diagnostic assay to provide simultaneous deep-sequencing information, based on > 500× average coverage, for all classes of mutations in 194 clinically relevant cancer genes. This study demonstrates that NGS assays are already capable of providing valuable predictive and prognostic information from clinical samples and can have an immediate impact on patient care.

Illumina Technology: HiSeq 2000, MiSeq

Rentsch C. A., Birkhauser F. D., Biot C., Gsponer J. R., Bisiaux A., et al. (2014) Bacillus calmette-guerin strain differences have an impact on clinical outcome in bladder cancer immunotherapy. Eur Urol 66: 677-688

BCG strain differences remain of enduring interest to urologists and TB vaccination specialists. The clinical efficacy, immunogenicity, and genetics of BCG treatment of 142 high-risk NMIBC patients with BCG Connaught or Tice were compared in a prospective randomized phase 3 trial. Genome sequencing of the BCG strains revealed putative genes implicated in differential clinical responses. The stronger immune response from BCG Connaught elicited a significantly better recurrence-free survival in patients with NMIBC. The genetic differences observed between the 2 BCG strains permit further scrutiny of the key determinants of effective BCG immunotherapy.

Illumina Technology: HiSeq 2000

Hopewell J. C., Parish S., Offer A., Link E., Clarke R., et al. (2013) Impact of common genetic variation on response to simvastatin therapy among 18705 participants in the Heart Protection Study. Eur Heart J 34: 982-992

Genetic associations with the lipid response to statin therapy have been reported with relatively modest effects and inconsistent study replication. The clinical efficacy of statins in reducing low-density lipoprotein (LDL) cholesterol thus remains controversial. The aim of this study was to investigate associations of common genetic variants with response to statin therapy through genotyping. The effects of multiple candidate genes on the lipid response to statins were assessed in up to 18,705 participants to substantiate current statin therapy practice guidelines. The value of common genetic variants for informing clinical decisions aimed at maximizing statin efficacy appeared to be limited.

Illumina Technology: 610K Quad Panel Array

Huang S., Holzel M., Knijnenburg T., Schlicker A., Roepman P., et al. (2012) MED12 controls the response to multiple cancer drugs through regulation of TGF-beta receptor signaling. Cell 151: 937-950

DRUG RESISTANCE

Not all drugs work as expected in all patients: a significant cohort of patients does not respond or responds inadequately to the applied therapy. Drug efficacy rates currently hover around 50%, and adverse drug reactions are believed to be the fourth leading cause of death in the US, costing ~$177 billion annually.[21] The development of resistance significantly reduces the chances of a drug candidate being translated into an approved therapy.[21] The use of NGS for the study of drug response and resistance is becoming increasingly popular. This technique has been tested in a variety of diseases, including multiple cancer types, such as ovarian cancer, tongue adenocarcinoma, and breast cancer.

Drug resistance can be pre-existing (ie, genetically programmed) or acquired. Acquired drug resistance is particularly common for cancers: most tumors are internally heterogeneic, ie, they harbor cells with different genomic profiles, and selective therapeutic pressure using a targeted therapy applied on one type of cell may provide a growth advantage to other types of cells, resistant to the drug.[21] The true causes of the genetic heterogeneity of cancers are not well understood, but genetic instability and chromatin remodeling are considered to be some of the most likely contributors.[21] Turner et al. suggest using NGS as a primary approach to track the tumor heterogeneity throughout the treatment, identify tumor resistance mechanisms, and disrupt them using a combination of adjuvant therapies.[69]

Biomarkers of Resistance

Acquired drug resistance is a major challenge in the chemotherapy of cancer.[70] A classic example of such resistance is a developed tolerance of breast cancers to tamoxifen, orchestrated through the natural downregulation of estrogen receptor, a receptor for estrogen and tamoxifen.[71] The most well-studied mechanism involves MDR observed in a variety of cancers. However, other mechanisms—such as methylation of the ABCG2 gene or deacetylation of histones—exist[72 73] and can be interfered with "epidrugs."

WGS, WES, and transcriptome sequencing have been used for the identification of drug-responsive and drug-resistant cell lines.[74] Jia et al. identified somatic SNVs and CNVs to characterize the changes in the resistant variants that were due to drug selection. The authors suggest that CNV changes may play a larger role than previously appreciated in the acquisition of drug resistance.[74] Microarray-based GWAS have been successfully used to identify a relationship between particular sequence variants and the drug efficacy for a number of common drugs, such as interferon-a, warfarin and steroid inhalers for asthma, clopidogrel (Plavix).[75] and therapeutic antibodies.[76]

Archiving biopsies or biological fluid samples throughout the treatment can deliver valuable information on drug response and resistance, as identified by NGS. This approach can also identify newly acquired resistant clones,[21] which may be highly informative in the development of combination therapies or companion diagnostics.[77] Cancer best demonstrates the importance of this approach: cancer cells are genetically unstable and have an ability to escape cytotoxic treatment through molecular adaptation.[78] Additionally, seemingly identical cancers can progress through different molecular mechanisms in different patients and have distinct responses to the same therapy.[79] Monitoring of tumor development has further informed the response to treatment.[3]

Li et al. identified a SNV explaining the resistance of 30% of schizophrenia patients to standard treatment with antipsychotic drugs. Better identification of treatment-resistant schizophrenia (TRS) is anticipated to increase the use of clozapine—the only drug approved for TRS—for treatment of this disorder. Use of this drug has long been limited, due to side-effects and difficulty in diagnosing the TRS phenotype.[80]

The Role of the Microbiome in Drug Metabolism and Resistance

The host microbiome is a significant factor in assessment of drug efficiency. More than 30 commercially available drugs are "biotransformed" by the gut microbiome.[81] This processing can be implemented in various ways. For example, microbes can secrete substances that serve as substrates for host enzymes processing a particular drug and thus reduce its efficiency. This process may also cause an increase in the drug toxicity, due to prolonged circulation in the blood. On the contrary, some drugs, especially antibiotics, can affect the shape and diversity of the microbiota. Computational microbiota-drug interaction models are now becoming available and are anticipated to advance therapy by changing the drug formulation or administration, or by formulating and transplanting the microbiome that would enhance the therapeutic effect.[82 83] Interestingly, transplanted microbiota per se can be used as therapy to treat multiple diseases, such as diabetes, obesity, diarrhea, inflammatory bowel disease, and acute gastroenteritis.[84]

Reviews

Dopazo J. (2014) Genomics and transcriptomics in drug discovery. Drug Discov Today 19: 126-132

Turner N. C. and Reis-Filho J. S. (2012) Genetic heterogeneity and cancer drug resistance. Lancet Oncol 13: e178-185

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Kasap C., Elemento O. and Kapoor T. M. (2014) DrugTargetSeqR: a genomics- and CRISPR-Cas9-based method to analyze drug targets. Nat Chem Biol 10: 626-628

This study introduces an assay, DrugTargetSeqR, to identify physiological targets of drugs. The assay combines Illumina RNA-Seq, computational mutation discovery, and CRISPR-Cas9–based genome editing. Genetically heterogenous cancer cells are treated with the drug of interest, and resistant clones are isolated and processed for RNA-Seq CRISPR-Cas9–based genome editing. Drug-based selection is used to determine which mutations are sufficient to confer resistance, followed by biochemical validation of the drug's direct target.

Illumina Technology: HiSeq 2500

Li J. and Meltzer H. Y. (2014) A genetic locus in 7p12.2 associated with treatment resistant schizophrenia. Schizophr Res

A substantial number of patients with schizophrenia have persistent psychotic symptoms refractory to at least 2 antipsychotic drugs and are thus termed treatment-resistant (TRS). A biomarker would be useful in identifying those patients with schizophrenia who respond favorably to clozapine treatment. A discovery GWAS was performed in 174 patients undergoing clozapine therapy in an investigational setting. Genetic heterogeneity was demonstrated in TRS patients with potential etiologic explanations for differential phenotypic presentation. The study underscores the possibility of distinct biologic and genetic schizophrenia subgroups with therapeutic implications for patients.

Illumina Technology: Human610-Quad (Infinium GT)

Mithraprabhu S., Khong T. and Spencer A. (2014) Overcoming inherent resistance to histone deacetylase inhibitors in multiple myeloma cells by targeting pathways integral to the actin cytoskeleton. Cell Death Dis 5: e1134

Recent evidence has indicated that combination of HDACi and PI is beneficial only in a subset of patients with advanced MM. This study examined the molecular signature associated with inherent resistance to HDACi in HMCL categorized as sensitive, intermediate, or resistant to HDACi. The authors used Illumina BeadArrays to evaluate gene expression profiles. They showed that, when HMCL and primary MM samples were treated with a combination of HDACi and agents targeting the signaling pathways, integral to the actin cytoskeleton, synergistic cell death was observed in all instances..

Illumina Technology: HT-12 Infinium BeadChip Arrays

Sun C., Wang L., Huang S., Heynen G. J., Prahallad A., et al. (2014) Reversible and adaptive resistance to BRAF(V600E) inhibition in melanoma. Nature 508: 118-122

Genetic variants in oncogenes may direct the choice of therapy for cancer treatment. In BRAF-mutant melanoma, treatment with B-Raf inhibitors may be effective; however, BRAF-mutant colon cancer tumors are intrinsically resistant to B-Raf inhibitors. In this study, the authors examined acquired epidermal growth factor receptor (EGFR) expression after the development of resistance to B-Raf or MEK inhibitors in melanoma tumors. Using whole-transcriptome Illumina sequencing, the authors determined the differential expression across melanoma cells before and after development of resistance to B-Raf inhibitors.

Illumina Technology: HiSeq 2000

Wagle N., Grabiner B. C., Van Allen E. M., Amin-Mansour A., Taylor-Weiner A., et al. (2014) Response and acquired resistance to everolimus in anaplastic thyroid cancer. N Engl J Med 371: 1426-1433

Therapies targeted at tumors with specific genetics may only be effective for a limited period until the tumor develops resistance to the treatment. Everolimus, an inhibitor of mTOR, is effective in treating tumors harboring alterations in the mTOR pathway. This case study describes a patient with metastatic thyroid carcinoma who exhibited an extraordinary 18-month response to everolimus. The authors used Illumina sequencing to characterize the tumor genome and normal blood genome before therapy, during therapy, and after acquired resistance. The resistant tumor genome revealed a mutation in the MTOR gene that confers resistance to allosteric mTOR inhibition.

Illumina Technology: HiSeq 2000

Arora V. K., Schenkein E., Murali R., Subudhi S. K., Wongvipat J., et al. (2013) Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. Cell 155: 1309-1322

The treatment of advanced prostate cancer has been transformed by novel antiandrogen therapies, such as enzalutamide. In this study, drug-resistant tumors were characterized by their gene expression (Illumina BeadArray HT12) and ChIP-Seq profiling (Illumina HiSeq 2000) to determine common features for resistance. The authors identified the induction of glucocorticoid receptor (GR) was sufficient to confer enzalutamide resistance, whereas a GR antagonist restored sensitivity.

Illumina Technology: Human HT-12 Infinium BeadChip Array, HiSeq 2000 (Sequencing)

Bardelli A., Corso S., Bertotti A., Hobor S., Valtorta E., et al. (2013) Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. Cancer Discov 3: 658-673

EGFR-targeted monoclonal antibodies are effective in a subset of metastatic colorectal cancers. However, all patients develop resistance through emergence of KRAS mutations. The authors studied the mechanisms of acquired resistance to cetuximab and panitumumab, 2 monoclonal antibodies that inhibit the signaling cascade initiated by EGFR, and in the clinical setting ameliorate the survival of patients with metastatic colorectal cancer. Using the Illumina HiSeq platform, the authors performed WGS and WES to characterize individual cancers and, through xenografts, evaluated the response to inhibitors.

Illumina Technology: HiSeq 2000 (Sequencing)

Jia P., Jin H., Meador C. B., Xia J., Ohashi K., et al. (2013) Next-generation sequencing of paired tyrosine kinase inhibitor-sensitive and -resistant EGFR mutant lung cancer cell lines identifies spectrum of DNA changes associated with drug resistance. Genome Res 23: 1434-1445

Somatic mutations in kinase genes are associated with the sensitivity of solid tumors to kinase inhibitors, but patients with metastatic cancer eventually develop disease progression. The authors studied acquired resistance (AR) in EGFR-mutant lung cancer drug-sensitive and drug-resistant cell lines using whole-exome, whole-genome, and whole-transcriptome Illumina sequencing. They identified somatic SNVs and CNVs to characterize the changes in the resistant variants that were due to drug selection. The authors suggest that CNV changes may play a larger role than previously appreciated in the acquisition of drug resistance and that resistance may vary across tumor cell populations.

Illumina Technology: HiSeq 2000 (whole-exome sequencing), Genome Analyzer $_{IIx}$ (whole-genome sequencing)

Kang Z., Yu Y., Zhu Y. J., Davis S., Walker R., et al. (2013) Downregulation of IGFBP2 is associated with resistance to IGF1R therapy in rhabdomyosarcoma. Oncogene

The insulin and insulin-like growth factor (IGF) signaling pathways are highly conserved and critical for growth and survival in many organisms. The IGF-1 receptor has been targeted for cancer treatment, but response rates are low with brief durations. This study sought to identify markers predictive for response and to understand the mechanisms of resistance. The authors used Illumina BeadChip Arrays for differential expression analysis across tumor cells with and without IGF-1 receptor antibody resistance. The authors suggest various drug combinations to enhance antibody activity and overcome resistance.

Illumina Technology: Human BeadChip Array

Umicevic Mirkov M., Cui J., Vermeulen S. H., Stahl E. A., Toonen E. J., et al. (2013) Genome-wide association analysis of anti-TNF drug response in patients with rheumatoid arthritis. Ann Rheum Dis 72: 1375-1381

Treatment strategies blocking tumor necrosis factor (TNF) have proven very successful in patients with rheumatoid arthritis (RA). However, a significant subset of patients does not respond, for unknown reasons. This paper describes a GWAS to identify genetic factors predicting anti-TNF outcome in patients with RA. The authors used Illumina HumanHap550 BeadArrays for genotyping 882 RA patients and identified 3 markers that showed consistent positive correlation with outcome over each of 4 replication cohorts.

Illumina Technology: HumanHap550(Duo/+/Quad+) (Infinium GT)

TECHNIQUES

WES delivers higher coverage than WGS (normally >100x vs. ~40x) on Illumina HiSeq 2000.[74]

A variety of NGS-based platforms have been developed to meet the growing demand of high-throughput genetic analysis in medical practice.[85] The most common applications are listed in Table 4.

Table 4: Applications of NGS Technology in Medical Practice.

Technique	Application
Whole-genome sequencing	Comprehensive detection of variations across the genome, capable of detecting all types of mutations (1 in Ong, F)
Whole-exome sequencing	Detection of variants in the coding regions of the genome, identification of actionable somatic mutations in cost effective manner where 1–1.5% of genome is sequenced (2 in Ong, F)
Whole-transcriptome sequencing	Expression of variant transcripts, fusion genes,[20] and allele-specific expression
Targeted (re)sequencing	Detection of mutations in well-known genes and gene panels that provides results that are more readily interpretable and of known clinical actionability
DNA methylation analysis	Detection of epigenetic modifications manifesting the interaction between the genome and the environment

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