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Mini-review

Application of next-generation sequencing in gastrointestinal and liver tumors

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ABSTRACT

Malignant transformation of normal cells is associated with the evolution of genomic alterations. This concept has led to the development of molecular testing platforms to identify genomic alterations that can be targeted with novel therapies. Next generation sequencing (NGS) has heralded a new era in precision medicine in which tumor genes can be studied efficiently. Recent developments in NGS have allowed investigators to identify genomic predictive makers and hereditary mutations to guide treatment decision. The application of NGS in gastrointestinal cancers is being extensively studied but continues to face substantial challenges. In our review, we discuss various NGS platforms and highlight their role in identifying familial mutations and markers of response or resistance to cancer therapy. We also provide a balanced discussion of the challenges that limit the routine use of NGS in clinical practice.

Introduction

Malignant transformation of normal cells is associated with the evolution of genomic alterations. This finding has led to the development of molecular testing platforms to find "Achilles heel" that can be targeted with novel molecularly-directed therapies. Molecular testing techniques have, therefore, evolved over the years and slowly transitioned from being a research concept to a modality that is readily available in routine clinic practice. The advent of next generation sequencing (NGS) has heralded a new era in clinical genomics. Several massive parallel sequencing approaches have dramatically decreased the cost of sequencing over the last decade [1]. These technologies resulted in a tremendous increase in throughput by sequencing millions of DNA fragments in parallel. The evolution of molecular diagnostic techniques paralleled by advances in drug development has translated into significant improvements in outcomes of certain patient subgroups. Notable examples include the introduction of ALK inhibitors in patients with lung cancer and ALK rearrangement [2], epidermal growth factor receptor (EGFR) inhibitors in patients with lung adenocarcinoma and EGFR mutations [3,4], or BRAF inhibitors in patients with melanoma who harbor a $BRAF_{v600E}$ mutation [5]. These advances have not been uniformly seen in all cancer types. Herein, we discuss the application of NGS in gastrointestinal and liver cancers and highlight recent advances, ongoing challenges and future directions.

Molecular testing platforms

The application of NGS in clinical settings requires careful interpretation of the output of NGS data in the context of "actionability". It is prudent to understand that the definition of "actionable" molecular alterations is based on several molecular, patient-specific and practical factors such as the availability of appropriate clinical trials or standard therapies. It is also critical to understand the performance characteristics and analytical validity of each sequencing method as well as the details of each variant considered as a basis for further clinical action.

There are several sequencing platforms and protocols (Table 1), but the basic steps start with library preparation, which usually involves fragmentation of nucleic acids into small fragments that are subsequently amplified and "bar-coded". The final actual "sequencing" step provides an output that is encoded by changes in fluorescent labels captured by an ultrasensitive camera or changes in pH captured by an ion-sensitive detector [6]. These data are then decoded into "reads" of nucleotide sequences strung together. These reads are subsequently aligned and mapped to their respective reference genomic regions. Variations from the reference genome can then be detected; this so called "variant calling" is achieved using computational algorithms that factor in the probability of each variant being a true variant based on the known sequencing errors and polymorphisms. Called variants have to be annotated afterward in order to infer the potential for functional significance. Several factors need to be considered for determining the potential functional impact of a mutation. These include the prevalence of a particular variant in databases such as COSMIC (Catalogue of Somatic

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Table 1
Next generation sequencing technologies.

NGS method	Detected molecular alterations	Description
Whole sequencing	Single nucleotide variants, Indels, Somatic copy number alterations	Sequencing of all protein coding regions (exons) of all genes
Targeted sequencing	Single nucleotide variants, Indels, and Somatic copy number alterations	Sequencing of exons of pre-selected list of genes; usually assembled in a "panel"
Whole genome sequencing	Single nucleotide variants, Indels, somatic copy number alterations, translocations and chromosomal rearrangements	Sequencing of all regions of the genome including exonic, intronic and intergenic regions
RNA sequencing	Expression levels of mRNA of different genes, detection of fusion transcripts from translocations, identification of splice variants and non-coding RNA	Sequencing provides a "snapshot" of the RNA transcribed from the genome at a give time point
Bisulfite sequencing: Methylseq and Reduced representation bisulfite sequencing	DNA methylation patterns	Utilizes the bisulfite reaction which converts cytosine residues to uracil while leaving 5-methylcytosine residues unconverted
ChIP-Seq	Identifying binding sites of DNA-associated proteins	ChIP (chromatin-immunoprecipitation) enriches cross-linked DNA–protein complexes selected using an antibody against the protein of interest
16S Ribosomal RNA sequencing	Bacterial phylogenetic classification for microbiome studies	Based on sequencing of hypervariable regions in the 16S to identify bacteria

Mutations in Cancer) and the location of a variant in reference to coding genes and its predicted function on the corresponding functional protein domains and three-dimensional structures [7].

The role of NGS in identifying predictive markers for response to cancer therapy

Several large projects such as The Cancer Genome Atlas (TCGA) have provided insight into genomic alterations that are prevalent in gastrointestinal (GI) cancers (Table 2) and can potentially be targeted through novel therapies [8–12]. Notable examples of progress that has resulted in identifying such genomic targets include treating gastrointestinal stromal tumors with *c-kit* mutations with imatinib [13] and gastric cancer with *HER2* amplification with trastuzumab (Table 3). Targeted therapies offer the potential for reducing side effects and potentially improving outcomes of treatment of GI cancer that harbor targetable alterations. Determining the feasibility NGS is a multifaceted process that involves evaluating its applicability to routine clinical practice, its yield, availability of targeted therapies and impact on patient outcomes. Several studies have, therefore, been launched and/or completed to further evaluate the feasibility of NGS in identifying patients with GI and other cancers who may benefit from targeted therapies [14–16]. A recent study has demonstrated that NGS is feasible in gastroesophageal cancer in clinical practice [14]. Eighty nine percent (50/56) of patients undergoing NGS had at least one actionable molecular alteration. The most prevalent alterations included cell cycle abnormalities (58%), *HER2* amplification (30%), *PI3KCA* mutations (14%), *MCL1* amplification (11%), *PTEN* loss (9%) and *MET* amplification (5%). These results are intriguing as several cell cycle inhibitors are currently in clinical development [17,18]. It is important to note, however, that NGS should not replace standard immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) techniques for HER2 testing in gastroesophageal cancer. Only 12/18 (66%) patients, positive for HER2 by IHC and/or FISH, demonstrated *HER 2* amplification by NGS. These results suggest that NGS should be added to IHC/FISH testing for HER2 overexpression or amplification rather than replace it. Recently, investigators from the University of Texas MD Anderson Cancer Center published their experience in evaluating the use of NGS to facilitate enrollment onto genomically-matched clinical trials [16]. Their cohort included 2000 patients, of whom 19% had gastrointestinal malignancy. The prevalence of actionable alterations including *KRAS* was 79%, 67% and 16% in pancreas, colorectal and gastroesophageal cancers, respectively. When *KRAS* mutations were excluded, the prevalence of actionable mutations was 31%, 16%, 16% and 11% in colorectal, esophageal, pancreatic and stomach cancers, respectively. In the whole cohort, 789 (39%) patients had at least one actionable mutation. However, only 83 patients (11% of those with actionable mutations; 4% of the total cohort) were enrolled in genotype-matched trials. The median time from consent to obtaining genomic test results was 26 days. The authors cited patient preference for local or non-investigational treatment, poor performance status, lack of trials or trial slots, insurance denial and/or trial ineligibility as the main challenges to clinical trial enrollment. This study is extremely valuable in understanding the current landscape of genomic testing and clinical trial enrollment and provides a platform to address the major challenges to the application of widespread genomic testing in cancer treatment. This study, however, did not report the outcome of patients who received targeted therapy. The outcome of patients with actionable molecular alterations that received targeted therapies has, however, been studies in the SHIVA trial [15]. The SHIVA trial is a randomized controlled open-label phase 2 trial that was launched in eight French academic centers to evaluate the efficacy of 11

Table 2
Notable molecular alterations in gastrointestinal cancers.

Tumor	Notable molecular alterations	References
Gastroesophageal	*TP53, CDKN2A, CCNE, CDK 4/6, CCND, EGFR, ERBB2, ERBB3, PI3KCA, PIK3R1, MCL1, PTEN, CDH1, JAK2, PD-L1/2, VEGFA, KRAS/NRAS* and *MET*	[14,37]
Small bowel	*IDH1, CDH1, KIT, FGFR2, FLT3, NPM1, PTEN, MET, AKT1, RET, NOTCH1 ERBB4, ERBB2, KRRAS, BRAF* and *FBXW7*	[38,39]
Colorectal	*APC, TP53, SMAD4, PIK3CA, PIK3R1,* PTEN, *KRAS, NRAS, BRAF, ARID1A, SOX9, FAM123B/WTX, ERBB2, IGF2 NAV2/TCF7L, ACVR2A, APC,* TGFBR2, MLH1, MSH3, MSH6, SLC9A9, *SMAD2, SMAD3* and *SMAD4*	[40]
Pancreas	*TP53, SMAD4, CDKN2A, ARID1A, ROBO2, KDM6A, PREX2, ERBB2, MET, FGFR1, CDK6, PIK3R3, PIK3CA, BRCA1, BRCA2, PALB2, KRAS, TGFBR2, BRAF, PREX2, MLL2, MLH2* and *MLH2*	[8]
Biliary	*TP53, KRAS, ERBB3, EGFR, ERBB2, ERBB4, FGFR2, PRKACA* and *PRKACB*	[9,10]
Hepatocellular	*TP53, CTNNB1, MET, PTEN, CDKN2A, AXIN1, PTEN, PIK3CA, KRAS, NRAS, MYC, MET, CCND2, RB1, ARID1A, ARID1B, ARID2, IRF2, NFE2L2, ERRFI1, RPSKA3* and *MLL3*	[41,42]