REVIEW

Molecular predictive markers in tumors of the gastrointestinal tract

Eirini Papadopoulou, Vasiliki Metaxa-Mariatou, Georgios Tsaousis, Nikolaos Tsoulos, Angeliki Tsirigoti, Chrisoula Efstathiadou, Angela Apessos, Konstantinos Agiannitopoulos, Georgia Pepe, Eugenia Bourkoula, George Nasioulas

Eirini Papadopoulou, Vasiliki Metaxa-Mariatou, Georgios Tsaousis, Nikolaos Tsoulos, Angeliki Tsirigoti, Chrisoula Efstathiadou, Angela Apessos, Konstantinos Agianni-topoulos, Georgia Pepe, Eugenia Bourkoula, George Nasiou-las, Department of Molecular Biology, GeneKor Medical S.A., 15344 Gerakas, Greece

Correspondence to: George Nasioulas, PhD, Department of Molecular Biology, GeneKor Medical S.A., 52 Spaton Ave, 15344 Gerakas, Greece. gnasioulas@genekor.com
Telephone: +30-210-6032138
Fax: +30-210-6032148

Abstract

Gastrointestinal malignancies are among the leading causes of cancer-related deaths worldwide. Like all human malignancies they are characterized by accumulation of mutations which lead to inactivation of tumor suppressor genes or activation of oncogenes. Advances in Molecular Biology techniques have allowed for more accurate analysis of tumors' genetic profiling using new breakthrough technologies such as next generation sequencing (NGS), leading to the development of targeted therapeutical approaches based upon biomarker-selection. During the last 10 years tremendous advances in the development of targeted therapies for patients with advanced cancer have been made, thus various targeted agents, associated with predictive biomarkers, have been developed or are in development for the treatment of patients with gastrointestinal cancer patients. This review summarizes the advances in the field of molecular biomarkers in tumors of the gastrointestinal tract, with focus on the available NGS platforms that enable comprehensive tumor molecular profile analysis.

Key words: Predictive biomarkers; Targeted therapy; Next generation sequencing; Gastrointestinal tract; Somatic mutations; Liquid biopsy

Core tip: Gastrointestinal cancers are among the leading causes of cancer morbidity and mortality worldwide. So far, various targeted agents associated with predictive biomarkers are available or are under development for the selection of treatment in patients with gastrointestinal cancer. Advances in high-throughput technologies such as next generation sequencing and the use of noninvasive

materials for tumor characterization, such as liquid biopsies, will facilitate tumor molecular profiling and lead to the establishment of further targeted treatment therapies.

INTRODUCTION

The comprehension of the importance of tumor biology has led to the development of new drugs that target specific molecules involved in carcinogenesis. The efficacy of such targeted therapies often depends on the presence or absence of gene alterations that encode for the protein-target or for proteins involved in the molecular pathway targeted by the specific medication. This targeted therapeutical approach is based on the tumor's molecular analysis in order to select patients with increased probability to respond to the treatment given. Advances in Molecular Biology techniques have permitted comprehensive tumor genomic profiling using new breakthrough technologies such as next generation sequencing (NGS)[1-3].

Nowadays, biomarkers are used in the management of patients with cancer and can be divided into predictive and prognostic. Prognostic biomarkers are defined as those that provide information on the possible outcome of cancer in a particular patient regardless of treatment. Predictive biomarkers provide information on the potential benefit of the administrated treatment (whether this relates to the tumor's volume shrinkage or survival). Predictive biomarkers can be used to identify subpopulations of patients that are likely to respond to a particular treatment[1]. They can be subdivided in positive and negative predictive biomarkers. The first are used for positive selection of patients who are likely to benefit from targeted therapy, whereas the latter for resistance prediction[1].

The number of genes involved in targeted therapy (predictive biomarkers), is increasing continuously. The simultaneous analysis of these biomarkers is feasible using molecular biology technologies that allow accurate, fast and cost effective genomic analysis with limited requirements concerning the quantity of the biological material used[1,4]. NGS has all the features required to carry out such analysis and provides simultaneous information on a large number of actionable alterations in tumor tissues and thus a more precise molecular characterization of the tumor. The massive amount of genetic information produced is the main advantage of this technology. However, it also constitutes its main challenge, requiring usage of appropriate software and bioinformatics tools, along with web-based tools for data analysis, management and interpretation[5].

The human gastrointestinal (GI) tract is an organ system which includes all structures between mouth and anus and is divided into upper (buccal cavity, pharynx, esophagus, stomach and duodenum) and lower (small and large intestine) GI tracts. GI cancers are complex diseases and refer to malignant conditions that affect the digestive system. The current review will focus on the advances in the field of molecular biomarkers and the application of high throughput technologies, in the most common tumors of the gastrointestinal tract.

Esophageal cancer

Esophageal cancer is one of the most aggressive malignancies with a rapidly increasing incidence rate in the recent decades. There are two predominant histological types: Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) of the distal esophagus and the gastroesophageal junction. Smoking and heavy alcohol consumption are associated with increased risk of ESCC, while gastroesophageal reflux disease and Barrett esophagus may of increase the risk of EAC[6].

TP53 mutations are identified in about 50% of esophageal cancers and are associated with poorer survival[7]. Apart from mutations in *TP53* ESCC and EAC seem to differ significantly in the genetic alterations pattern. Agrawal *et al*[8] using NGS reported a substantial disparity in the spectrum of mutations, with more insertions/deletions in ESCCs, A:T>C:G transversions in EACs, and C:G>G:C transversions in ESCCs. Inactivating mutations of *NOTCH1* are identified in about 20% of ESCCs but not in EACs. Somatic aberrations in EACs are mainly identified in the Wnt, cell cycle and Notch pathways[9,10]. A number of genes that can be used as predictive markers for targeted therapy have been explored for somatic mutations in esophageal adenocarcinoma, including genes of the RAF/MEK/ERK (MAPK) kinase pathway such as *EGFR, BRAF, KRAS, PIK3CA*[11]. However the reported frequency of somatic mutations identified appears to be low and this is obvious when accessing data from the Catalogue of Somatic Mutations in Cancer database (COSMIC, cancer.sanger.ac.uk) (Figure 1), which is currently the most comprehensive global resource accessing the world literature on somatic mutations in human cancer[12]. In a recent study, NGS-based comprehensive genomic profiling was used to analyze ESCC and EAC tumors[13]. The analysis showed that the esophageal histotypes differ significantly in genomic alterations profile, with *KRAS* and *ERBB2* far more frequently altered in EAC compared to ESCC. In contrast, genes of the mechanistic target of rapamycin (MTOR) pathway (*PIK3CA* and *PTEN*) and *NOTCH1* are more frequently altered in ESCC compared to EAC. They also have different amplification patterns (Figure 2).

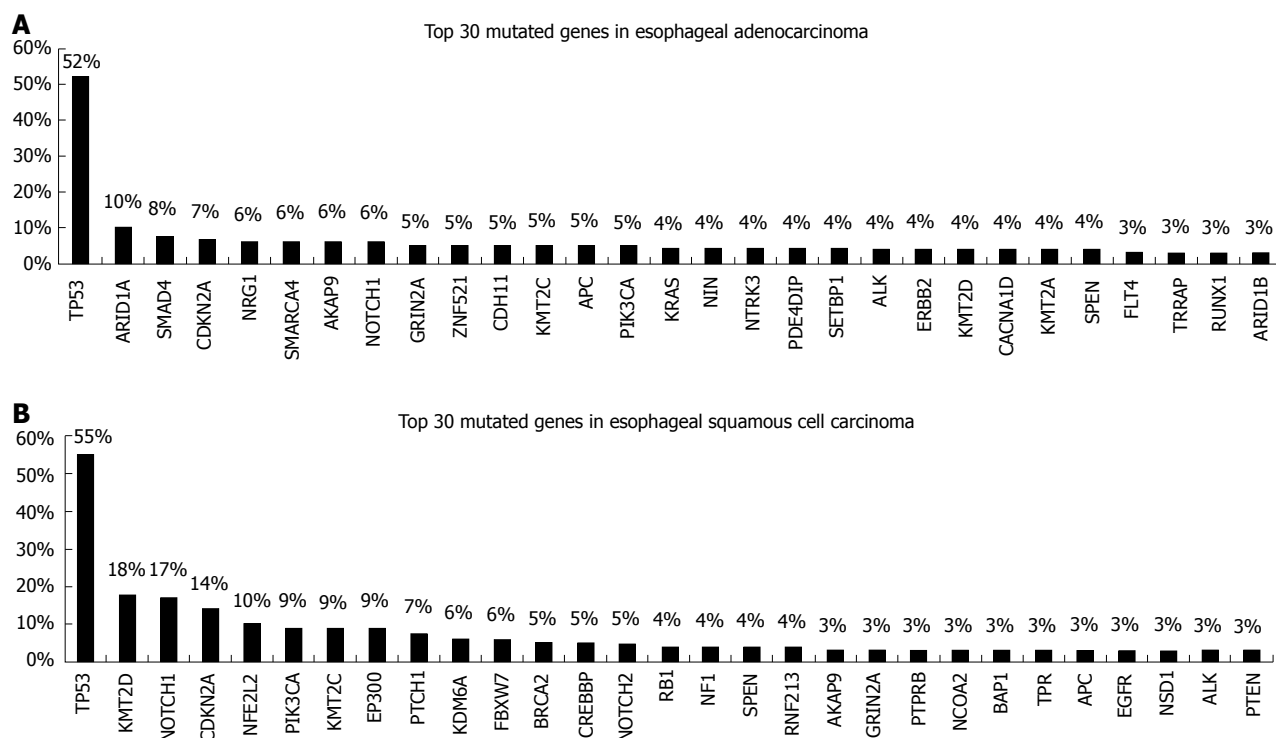ESCC and EAC also differ in the gene amplification

A

Top 30 mutated genes in esophageal adenocarcinoma

B

Top 30 mutated genes in esophageal squamous cell carcinoma

Figure 1 Bar chart showing the most frequently mutated genes in esophageal cancer according to catalogue of somatic mutations in cancer database. In the Y-axis the percentage of observed mutation frequency is represented. In the X-axis the most frequently mutated genes are listed. A: Top 30 mutated genes in esophageal adenocarcinoma; B: Top 30 mutated genes in esophageal squamous cell carcinoma.

and/or protein (over)expression of the receptor tyrosine kinases (RTKs) EGFR and HER2 making them possible prognostic markers and as therapeutic targets[7,14]. EGFR is frequently overexpressed in ESCCs, while HER2 overexpression occurs mainly in EACs. Thus the trastuzumab-platinum regimen is currently used for the 15% of the EACs patients that test positive for HER2 (ERBB2) amplification or overexpression[13,14].

Numerous preclinical studies addressed EGFR and HER2 inhibition in esophageal cancer cell lines and there are various phase II/III clinical trials testing EGFR, HER2, and VEGF targeting therapies for esophageal cancer[7,15]. However, the results obtained to date do not allow the use of these agents in clinical practice. Upon trial completion several clinical studies have concluded, that in order to select patients who will respond to RTK-targeted therapy, there is a need for molecular patient stratification before treatment.

In a disease with historically poor outcomes and limited options, comprehensive genomic profiling of relapsed and refractory cancers, including distinct evaluation for EAC and ESCC has led to promising information suggesting targeted therapies for future consideration.

Gastric cancer

Gastric cancer (GC) develops from the inner lining of the stomach and is a very aggressive malignancy, with poor prognosis and very high cancer related mortality. The high mortality rate is largely due to the late stages of cancer diagnosis and to the lack of effective medical treatment for advanced stages of this disease[16,17]. The majority of these cancers are adenocarcinomas and can be further classified as diffuse (poorly differentiated) or intestinal (well-differentiated) types that have distinct molecular profiles[16,17].

Concerning the causes of GC, it can be viewed as a multifactorial disease since many inherited and environmental factors play a role in its development. Infectious agents such as Helicobacter pylori and EBV, dietary habits and the genetic background are considered as causative agents[17].

Given the variety of causes of the disease, it is not surprising that these tumors present a high level of biological heterogeneity, with distinct molecular profile for each patient. Genetic and epigenetic alterations play important role in GCs therefore, targeted therapy based on the biology of the individual patient could improve treatment outcome[17-19].

ERBB2 amplifications occur frequently in gastric tumors (2%-27%)[12,20]. Trastuzumab, a monoclonal antibody against HER2/neu receptor, was the first targeted agent to be used in the treatment of ERBB2-positive advanced gastric and gastroesophageal junction (GEJ) adenocarcinoma[20]. Several molecular targeted agents associated with a survival benefit in other cancer types are now under clinical investigation for the treatment of gastric cancer, including inhibitors of EGFR, MET, FGFR, VEGF, and PI3K[18,20]. Additionally, *CDH1* gene mutations at the somatic level are considered of prognostic significance[19].
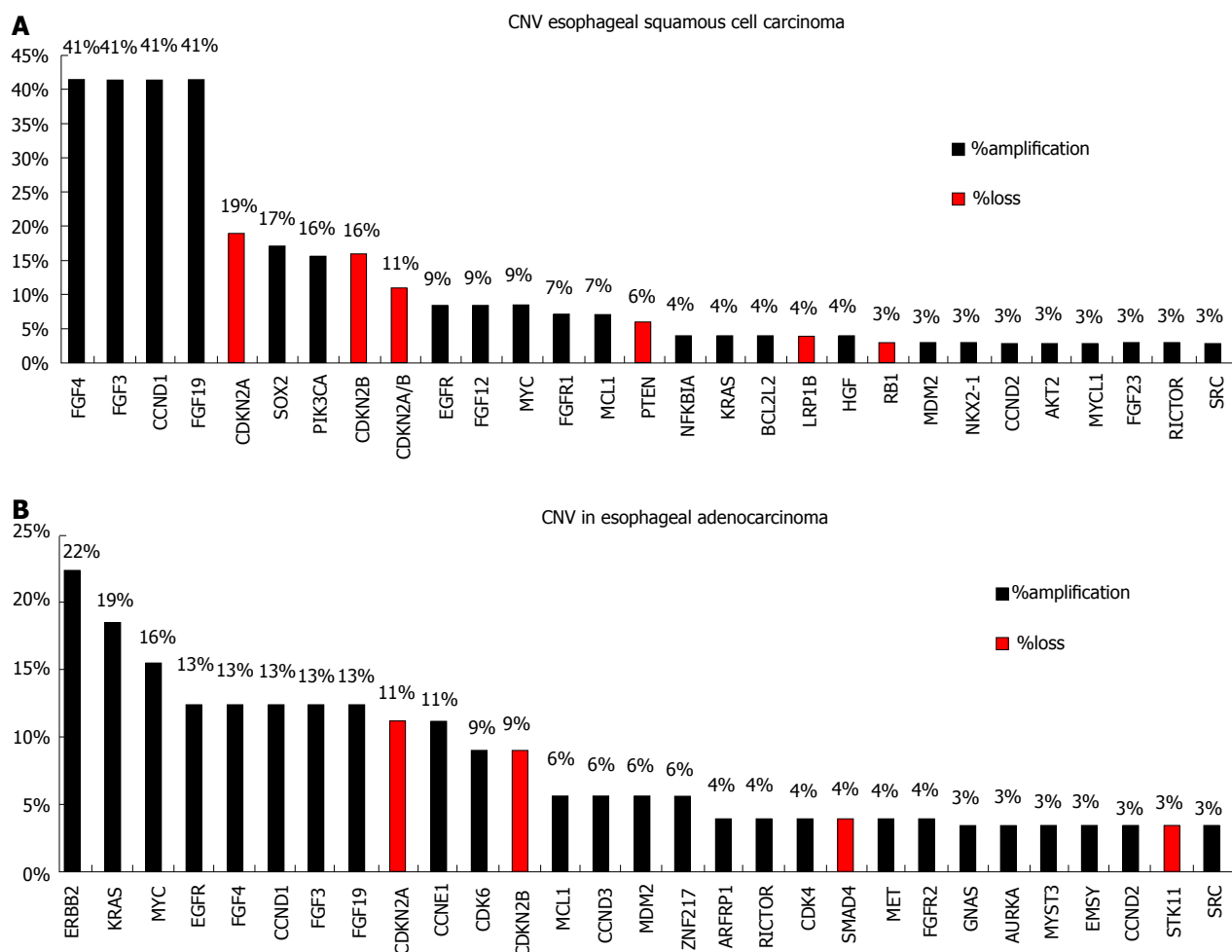
Several studies have investigated gastric cancer's

Figure 2 Bar chart showing the copy number variations in esophageal cancer according to the study performed[13]. In the Y-axis the percentage of CNV frequency is represented. In the X-axis the most frequently altered genes are listed. A: Genes most commonly affected by CNV (amplification or loss) in esophageal adenocarcinoma; B: Genes most commonly affected by CNV (amplification or loss) in esophageal squamous cell carcinoma. CNV: Copy number variation.

molecular profile using whole genome as well as targeted NGS approaches[19,21-23]. The presence of somatic mutations and copy number variations (CNV) in many cancer driver genes has been revealed. Among the cancer genes frequently mutated in gastric cancer *P53, ARD1A, CDH1, PIK3CA, APC, CTNNB1, ERBB3, ATM, KRAS* are the most important prognostic and/ or predictive markers (Figure 3)[12]. Consequently, molecular profile-directed therapy seems to be a promising strategy for the improvement of standard chemotherapy effectiveness.

CNVs have been observed for HER2, FGFR2, and MET that represent viable treatment targets for which therapeutics are already approved or are currently under investigation[24] (Figure 4).

The high heterogeneity of these tumors triggered scientists to attempt their molecular characterization. In a study conducted by The Cancer Genome Atlas, molecular classification four major genomic subtypes of gastric cancer were defined: EBV-infected tumors; MSI tumors; genomically stable tumors; and chromosomally unstable tumors[21].

In a recent study, Li *et al*[19], using whole genome

NGS data were able to classify gastric cancers into regular (86.8%) and hyper-mutated (13.2%) subtypes based on mutation burden. Additionally, in the "regular" mutated cohort a further classification, using 40 significantly mutated genes, could be obtained, separating the patients to S1 and S2 subtypes with distinct prognostic outcomes.

Gastrointestinal stromal tumors

Gastrointestinal stromal tumors (GISTs) are rare tumors of the gastrointestinal tract. They are mesenchymal in origin and are characterized by overexpression of the KIT protein[25]. Morphological diagnosis based on microscopic examination is the standard for GIST diagnosis. They occur anywhere within the GI tract, but they are most common in the stomach (60%) or small intestine (30%)[26]. Their diagnosis is based on the expression of the transmembrane tyrosine kinase (TK) receptor, KIT, since 95% of GISTs express CD 117 antibody. In 80% of the cases, somatic mutations in the *cKIT* gene are observed, resulting in constitute receptor activation. Additionally, in 5%-10% of the cases without *cKIT* mutations, the TK receptor *PDGFRA* is
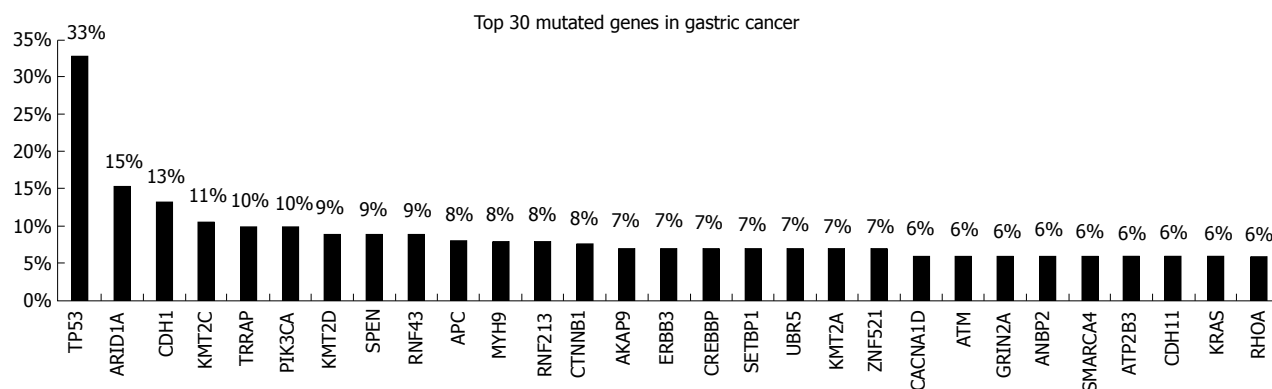
Figure 3 Bar chart showing the most frequently mutated genes in gastric cancer according to catalogue of somatic mutations in cancer database. In the Y-axis the percentage of observed mutation frequency is represented. In the X-axis the genes are listed.
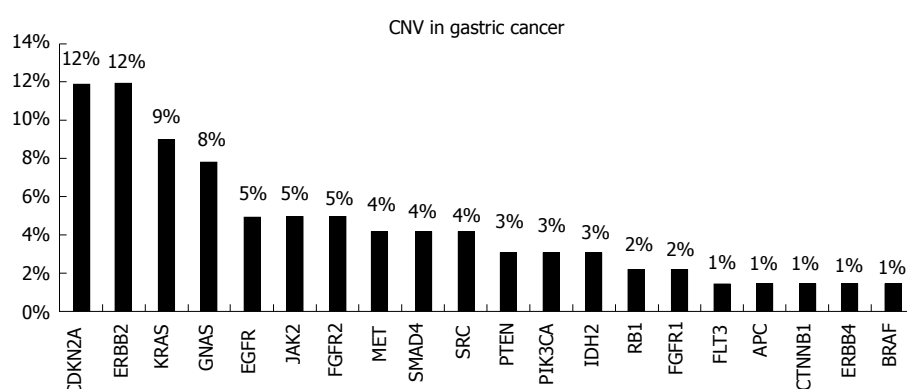

Figure 4 Copy number variation in the most important treatment targetable genes in gastric cancer. In the Y-axis the percentage of observed CNV is represented. In the X-axis the genes are listed. CNV: Copy number variation.

mutated[27]. The mutation spectrum of these tumors is very limited as we observe in COSMIC database (Figure 5)[12]. Tyrosine kinase inhibitors (TKIs) like imatinib, sunitinib, and more recently regorafenib, have proven effectiveness in suppressing the growth of metastatic GISTs, allowing patients to live far longer than during the previous era of ineffective chemotherapy[28-30]. The response to targeted therapy with TKIs is mainly dependent on the presence and type of mutation. Patients with mutations in exon 11 of the *cKIT* are highly responsive to imatinib, while the presence of a mutation in exon 9 of this gene implies intermediate response rates and necessitates a double dose of drug administration. Furthermore, resistance mutations to imatinib are also observed in the *cKIT/PDGFRA* genes. These mutations can be present in the primary tumor or arise as a result of the drug administration (secondary mutations)[29].

Colorectal cancer

The cancer of colon and rectum (colorectal, CRC) is the third most common cancer worldwide with 95% of these tumors being classified as adenocarcinomas. It's a leading cause of cancer related deaths; however, colorectal cancer mortality is declining in the last decades, mainly due to early diagnosis and the presence of new therapy strategies[31,32]. This malignancy is one of the

first paradigms of the benefits that can be derived from the application of personalized treatment in cancer therapy[33-35].

Genetic alterations in colorectal cancer include mainly single-base substitutions (SBS). Nevertheless, small insertions and deletions (indels), amplifications, homozygous deletions and translocations can also be observed[36].

Five hundred and seventy-two cancer relevant genes are included in the Catalogue of Somatic Mutations in Cancer (COSMIC, cancer.sanger.ac.uk)[12]. Somatic mutations in CRC cancer are observed in the majority of these genes. The pattern of genomic alterations was identified through Massively Parallel Sequencing studies, revealing the inter- and intra-tumor genetic heterogeneity of these tumors[37-39]. Apart from single base substitutions, gene amplifications are also observed (Figure 6). Mutations in many important biologic pathways occur. In Table 1 the frequency of mutations in important molecular signaling pathways and the related therapies are represented. The gene mutation frequency is calculated using data from samples analysed by whole genome screening in the COSMIC database. The information concerning the therapies targeting each pathway was retrieved from MyCancer Genome knowledge database (www.mycancergenome.org/), that provides reliable information concerning
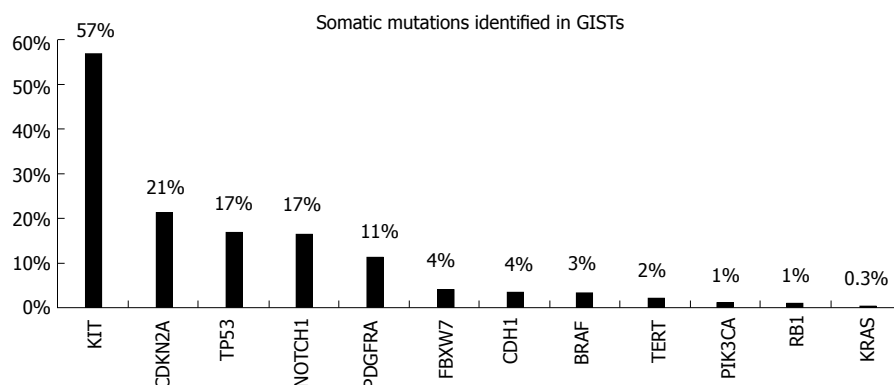
Somatic mutations identified in GISTs

Figure 5 Bar chart showing the most frequently mutated genes in gastrointestinal stromal tumors according to catalogue of somatic mutations in cancer database. In the Y-axis the percentage of observed mutation frequency is represented. In the X-axis the genes are listed. GIST: Gastrointestinal stromal tumors.
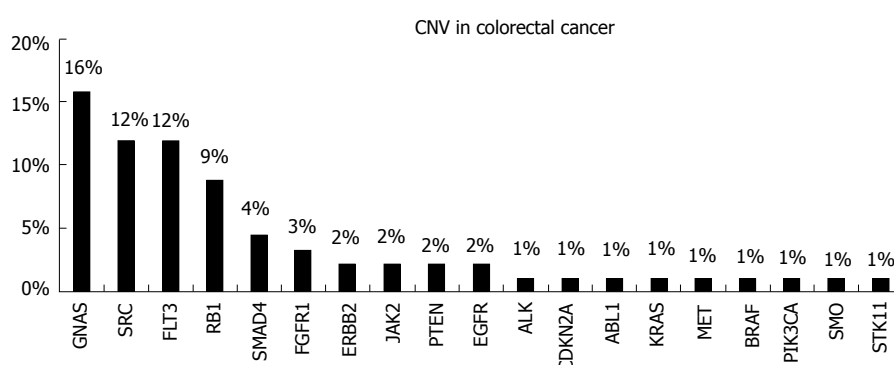
CNV in colorectal cancer

Figure 6 Copy number variation in the most important treatment targetable genes in colorectal cancer. CNV: Copy number variation.

important cancer related genes and their correlation with treatment options (Table 1)[40].

The *RAS* proto-oncogenes (*HRAS, KRAS* and *NRAS*) encode a family of highly homologous proteins. They participate in a signal transduction cascade, namely the RAS/RAF/MEK/ERK pathway, which regulates the growth and survival properties of the cells. They are controlled by extracellular signals transmitted by the transmembrane receptor tyrosine kinase (TK), EGFR[34]. This TK and the RAS/RAF/MEK/ERK pathways it controls, play an important role in colorectal carcinogenesis, making it a good target for biological therapy of this disease.

Two monoclonal antibodies were designed as effective inhibitors of EGFR. Cetuximab (Erbitux, Merck KgaA, Darmstadt, Germany) is a chimeric mouse/human antibody, and Panitumumab (Vectibix, Amgen Thousand Oaks, CA, United States), is a fully human antibody[33-35]. They both target the extracellular domain of the EGFR protein and compete with ligands, blocking ligand induced intracellular signal transmission. However, anti-EGFR treatment is not effective in patients harboring activating mutations in genes that participate in the intracellular transduction RAS/RAF/MEK/ERK pathway. This is due to the constitutive, independent of ligand, activation of the mutated proteins[33].

In total, activating mutations in the *RAS* genes, mainly in codons 12, 13 or 61, occur in approximately 20% of all human cancers. Mutations in *KRAS* account for about 85% of all *RAS* mutations in human tumors, *NRAS* for about 15%, and *HRAS* for less than 1%[41,42]. Which particular *RAS* gene is mutated seems to be tumor specific. Colonic, pancreatic and lung cancers have high frequencies of *KRAS* mutations[41,42].

Acquired mutations in *KRAS* and *NRAS* are commonly used to identify colorectal cancer patients who are unlikely to benefit from anti-EGFR therapy. Approximately 40% of colorectal cancer tumors harbor mutations in the *KRAS* gene, with the majority of the mutations occurring in codons 12, 13 and 61. In 5% of the colorectal cancer cases a mutation occurs in the *NRAS* gene[35,41].

Another important gene of the RAS/RAF/MEK/ERK pathway is *BRAF*. Mutations in the *BRAF* gene (exons 11 and 15) have been detected in about 12% of colorectal cancers and are mutually exclusive with *RAS* mutations[12,43,44]. The *BRAF* activating aberrations, result in constitutive BRAF kinase activity, ERK signaling, proliferation and transformation[44]. The majority of *BRAF* mutations are observed in exon 15 (codon 600) and a minority of mutations are observed in exon 11[44,45].

Several studies have reported that patients with metastatic CRC (mCRC) that harbor *BRAF* mutations do not respond to the anti-EGFR antibody agents cetuximab or panitumumab[43,46,47]. However it is unclear if the presence of *BRAF* mutations in CRC cancer can be

Table 1 Overall gene mutation frequency in each molecular signaling pathway

Biologic pathway	Frequency of mutation in genes involved in each pathway	Therapies that target the pathway
Beta-catenin/WNT signaling	75%	FZD, GSK inhibitors
Cell cycle control	68%	CDK, CDK1, CDK2, CDK4/6 inhibitors
Receptor tyrosine kinase/growth factor signaling	67%	Therapeutic antibodies/tyrosine kinase inhibitors
MAP kinase signaling	61%	BRAF, ERK, MEK AND SRC inhibitors
PI3K/AKT1/MTOR	52%	Allosteric mTORC1 inhibitors/mTORC1/2 catalytic inhibitors
DNA damage/repair	48%	PARP INHIBITORS
TGFbeta signaling	37%	TGFBR1 inhibitors
Chromatin remodeling/DNA methylation	32%	DNMT inhibitors, Histone deactylase
Immune checkpoints	26%	Anti-CTLA4 antibodies, anti-PD-1 antibodies, Anti-PD-L1 antibodies, Immunotherapies
JAK/STAT signaling	23%	JAK inhibitors
Hedgehog signaling	12%	SMO inhibitors

The information concerning therapies that target each molecular pathway was retrieved for MyCancer Genome Site (Available from: URL: http//www.mycancergenome.org/).

used as a predictive marker or if it has only a prognostic value, independent of treatment, since different studies arrive at controversial conclusions concerning its clinical significance[45,48].

The *PIK3CA* gene encodes the catalytic subunit of phosphatidylinositol 3-kinase while belongs to a family of lipid kinases. These kinases regulate a diverse range of cellular processes including cell proliferation, adhesion, survival, and migration[49]. Mutations in *PIK-3CA* stimulate downstream AKT-mTOR signaling pathways, thereby promoting growth-factor independent growth, cell invasion and metastasis. *PIK3CA* mutations have been reported in multiple malignancies, including approximately 25% of gastric, 4% of lung, 25% of breast, and 20% of colorectal cancers[50]. The majority (80%) of *PIK3CA* mutations cluster in 2 "hotspot" regions, the helical domain (exon 9) and the kinase domain (exon 20). Concomitant *PIK3CA* mutations in exons 9 and 20 seem to be linked to significantly worse cancer-specific survival[51]. *PIK3CA* mutations may also be associated with clinical resistance to EGFR-targeted monoclonal antibodies, but there have been conflicting results[52-55]. A meta-analysis comprising 864 patients, from 11 studies, with colorectal cancer treated with cetuximab or panitumumab-based therapy showed that *PIK3CA* mutations, particularly in exon 20, are significantly associated with worse response and shorter progression-free and overall survival[51]. Somatic *PIK3CA* mutations have also been associated with superior colorectal cancer-specific survival in patients who regularly intake aspirin after diagnosis[56]. *PIK3CA* activating mutations may also predict sensitivity to inhibitors of the PI3K-AKT-mTOR pathway[57]. Inhibitors of mTOR, PI3K, and AKT, alone or in combination with other therapies are in clinical trials in solid tumors[58,59].

A number of rare gene mutations occurring in the PI3K/AKT/mTOR pathway are potentially actionable in colorectal cancer. PTEN is a key negative regulator of the PI3K pathway. *PTEN* gene mutations occur in about 5% of colorectal cancers[12,60,61]. *PTEN* inactivating mutations and *PTEN* loss have as a consequence the

upregulation of the PI3K/ AKT pathway[54,61].

Currently, the prognostic and predictive significance of *PTEN* mutations or *PTEN* loss of expression is under investigation. In retrospective studies, *PTEN* loss was associated with decreased sensitivity of colorectal cancer tumors to anti-EGFR antibodies[60-62]. Preclinical data and *in vitro* studies suggest that it may be associated with sensitivity to PI3K and mTOR inhibitors. Based on these data, several PI3K and mTOR inhibitors are currently in clinical trials for the treatment of patients with PTEN-deficient cancers[61,63].

AKT (Protein kinase B, PKB) is a serine/threonine kinase that is encoded by three genes AKT1, 2 and 3. Somatic mutations in the *AKT1* gene occur in colorectal cancer in about 1% of the cases according to the COSMIC database[12]. The only mutation observed is the activating mutation E17K, which is also observed in other types of cancer[64]. AKT1 is a critical component of the PI3K/AKT/mTOR pathway, thus it has become an attractive target for therapeutic intervention[49,65]. *AKT1 E17K* mutations have also been associated with primary resistance to cetuximab[66].

In colorectal cancer, DNA mismatch repair (MMR) system deficiency occurs frequently and leading to microsatellite instability (MSI). These are small changes in the DNA sequence that occur during DNA replication and are usually additions or deletions of one or two nucleotide bases[67,68]. This phenomenon is most common in areas of the genome that contain repetitive DNA sequences with a repeat unit, from one to four bases, and are known as Microsatellite regions[67,69]. The presence of microsatellite instability (MSI High) is a good prognostic marker[67,70]. It is found in 90% of cases of tumors arising patients with hereditary Lynch syndrome and in 10%-15% of the sporadic cancers[71]. Sporadic MSI-H tumors can be distinguished from the hereditary ones through somatic mutation analysis of the *BRAF* gene or loss of *MLH1* expression[72,73]. Somatic mutations in the *BRAF* gene occurs only in sporadic MSI-H tumors but not in Lynch-associated CRC cancers. Similarly, *hMLH1* promoter methylation rarely occurs in

Lynch syndrome-associated cancers, while is common in sporadic MSI-high cancers[73].

Recent studies have indicated that MSI high tumors, both sporadic and hereditary, are less aggressive and are related with low probability of lymph node and distant recurrences[70]. In addition they respond differently to chemotherapy, since they are less sensitive to Topoisomerase inhibitors and to the treatment with 5-fluorouracil[74-76]. Additionally, it has been proposed that MMR-deficient tumors are more responsive to PD-1 blockade than the mismatch repair-proficient tumors[77].

Gene expression profiling (GEP) is an emerging tool which aims to identify differentially expressed subsets of genes (gene signatures) in groups of patients with distinct clinical outcomes. Several commercial GEP tests are currently available for stage II/IIIa colorectal cancer patients. Oncotype DX® Colon Cancer Assay (Genomic Health, Inc., Redwood City, CA) and ColoPrint (Agendia, BV, Amsterdam, Holland) are the most promising gene signature tests[78]. Both tests provide a risk of recurrence, but OncotypeDx has the advantage of being applicable to Formalin Fixed Paraffin Embedded (FFPE) tissue for analysis, while ColoPrint requires fresh tissue which is not easily available. Oncotype DX® Colon Cancer Assay is a quantitative reverse transcription polymerase chain reaction (RT-qPCR) assay on RNA extracted from FFPE tumor tissue, used to assess risk of recurrence in stage II colon cancer patients at three years after surgery[79-81]. The test uses gene expression profiling of 12 genes that include seven prognostic genes and five reference genes, in order to provide a Recurrence Score (RS). The RS allows patients and physicians to determine the risk of developing a distant metastasis. In a retrospectively performed study by Yothers *et al*[82], RS was the strongest predictor of disease recurrence independent of other factors, such as T-stage, mismatch repair status, number of nodes examined, tumor grade, and lymphovascular invasion. The greatest utility of this test seems to be in the prediction of recurrence risk in T3, mismatch repair-proficient (MMR-P) stage II colon cancer patients[82]. However, it has also been validated in stage III patients with very promising results[83]. The continuous RS predicted recurrence as well as disease free survival (DFS) and overall survival (OS) in all three patient subgroups (stage II, IIIA/B, and IIIC). The use of this assay could lead to overall reduction in adjuvant chemotherapy use in this subgroup of stage II/III colon cancer patients[83].

LIQUID BIOPSY

Until recently, the best material for somatic mutation analysis was considered formalin fixed paraffin embedded (FFPE) tumor tissue. FFPE tissue is a widely available material, easy to use and maintain. In addition, the cancer tissue can be selected and mutation analysis can be performed without contamination by normal tissues[42]. This increases the sensitivity of mutation detection assays which is very important because, due to tumor heterogeneity, somatic mutations can sometimes be present at a very low percentage. However, FFPE tissue material also has several disadvantages[84,85]. First of all in some cases it is not available. This is the case of non-operable tumors. Furthermore, the examination of a limited tumor area present in a paraffin block doesn't take into account tumor molecular heterogeneity and does not necessarily reflect the molecular profile of other tumors or metastasis that are eventually present in the patient's body[85]. Additionally, the genetic material obtained, due to the paraffinization process, is sometimes of very bad quality and not suitable for molecular analysis[86]. Most importantly, tumor molecular profile is altered mainly following therapy and those alterations cannot be detected by analyzing the primary tumor material[87].

Nowadays, the presence of cell-free tumor derived nucleic acids (ctDNA/ctRNA) in cancer patients body fluids (plasma, serum, Broncho-alveolar, urine, stool, *etc.*) is well documented[84]. The term Liquid Biopsy has emerged indicating the use of these noninvasive materials for tumor characterization. The mutation status detected in a liquid biopsy reflects the status present in the patient's tumor. Furthermore Liquid Biopsy analyses take into account intra-tumor or inter metastatic heterogeneity and could eventually detect more tumor alterations compared with the analysis of a specific area in a FFPE tissue[83,84]. A variety of sensitive methods can be used for the detection of ctDNA in plasma samples, including digital PCR, Real time PCR, Arms PCR and NGS[84].

The utility of liquid biopsy analysis has been proven in many studies that used ctDNA for the detection of tumor specific alterations in plasma with prognostic and/or predictive significance[87-98]. A liquid biopsy analysis can be performed before treatment as well as for patients monitoring during therapy. It is also very helpful in the detection of secondary mutations that arise due to targeted therapy. The detection of secondary mutations in plasma can modify the treatment strategy for those patients (Table 2).

NGS METHODOLOGIES

NGS is a general term referring to all post-Sanger sequencing technologies that are able to massively sequence millions of DNA segments[99,100]. The goal of these technologies is to increase sequencing capacity and speed at a lower cost. Furthermore, the sensitivity obtained is superior to that of the conventional sequencing technology, making possible the detection of mutations that are present at very low percentages in a background of normal DNA, which is very important for somatic mutation detection. Currently the most widely used platforms are those offered by Illumina, Inc. (United Sates); Thermo Fisher Scientific, Inc. (United

Table 2 Gene mutations identified in ctDNA of patients with tumors of the gastrointestinal tract and their correlation with possible clinical implications

Gene	Mutation type	Tumor type	Possible clinical implications	Ref.
KRAS/NRAS	Point mutation/amplification	Colorectal/pancreatic cancer	Resistance to anti-EGFR therapy/sensitivity to MEK inhibitors	[88-90]
BRAF	Point mutation	Colorectal cancer	Resistance to anti-EGFR therapy/sensitivity to MEK inhibitors	[88,91]
MET	Amplification/alteration	Colorectal/esophageal cancer	Resistance to anti-EGFR therapy/sensitivity to MEK inhibitors	[92-94]
HER2	Amplification	Colorectal/gastric cancer	Resistance to anti-EGFR therapy/sensitivity to Anti HER2 inhibitors	[95,96]
EGFR	Point mutation	Colorectal/pancreatic cancer	Panitumumab	[97,98]
PIK3CA	Point mutation	Colorectal/pancreatic cancer	mTOR inhibitor	[89]
Ckit	Point mutations	GISTS	Imatinib or dose escalation or alternative TKIs	[99,100]
PDGFRA	Point mutations	GISTS	Imatinib or dose escalation or alternative TKIs	[99,100]

TKI: Tyrosine kinase inhibitors; GIST: Gastrointestinal stromal tumors; EGFR: Epidermal growth factor receptor; mTOR: Mechanistic target of rapamycin.

Sates) and Roche Holding AG (Switzerland)[101-103]. The first NGS platform was created by Roche and used emulsion PCR (emPCR) to clonally amplify the fragments that are then sequenced *via* sequencing-by-synthesis (SBS) technology[101]. The Illumina platform is currently widely used in the NGS market and involves bridge amplification, of a solid surface-bound DNA, to clonally amplify the fragments that are then sequenced using SBS chemistry[102]. Unlike the previous two technologies, the Life technology platform uses Ion semiconductor sequencing, instead of fluorescence based sequencing, detecting the protons released as nucleotides are incorporated during synthesis[103,104].

The past years have seen an accelerating outbreak of publications in which NGS is applied for a variety of goals such as full-genome resequencing or more targeted mutation detection. Worldwide collaborative efforts, such as COSMIC database, International Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA) project, enabled to catalogue NGS data of thousands of cancer genomes across many disease types[105,106]. Targeted NGS, involving gene panels, is a quicker and cost effective alternative to whole genome sequencing or exome sequencing. Targeted NGS panels for somatic mutation detection include actionable cancer genes and allow the determination of the patient's tumor molecular profile. The goal of their use is to increase the percentage of patients with detected actionable alterations and with copy number data, allowing them to be included in clinical trials[107-109].

Such panels are currently available or can be custom made. They exhibit high rates of sensitivity, specificity and repeatability; therefore they are optimal for diagnostic use. Benchtop NGS sequencers are now offered by both Illumina (MiSeq) and Thermo Fisher (PGM™ and Ion Proton™). The availability of the equipment required and the cost effectiveness of the analysis allows its implementation in local specialized laboratories[108,109]. However, the reliability of these tests should be reassured. Thus, NGS performing laboratories should have specialized personnel and equipment which will provide adequate data analysis management and interpretation with the aid of appropriate software and bioinformatics tools. Importantly, these tests should be operated under the guidelines of a quality assurance system[110,111].

Concerning the selection of the appropriate sequencing platform, it should be based on the individual laboratory's needs. All NGS platforms have advantages and disadvantages and the choice of the platform used should be based on the application for which it is required. For example, the MiSeq platform (Illumina) has lower error rates especially in the homo-polymer regions compared to both Ion Proton and PGM (Life Technology). However it requires higher DNA concentration and quality, which is not always available when the starting material is FFPE tissue. On the other hand, the NGS platforms offered by Thermo Fisher provide a fast and cost-effective sequencing solution with good analytical performance. Additionally, they more compatible with low DNA concentrations and partially degraded poor quality DNA from FFPE samples[107-109,112]. Consequently, they provide an attractive option of clinical utility for the detection of cancer hotspot mutation analysis.

CONCLUSION

This review summarizes the use of biomarkers in the most common cancers of the GI tract. They are used for positive selection of patients who are likely to benefit from targeted therapy or for resistance prediction. Biomarker based targeted treatment is established in a subset of patients with gastrointestinal cancer. Meta-analysis studies have shown that biomarker based treatment is a promising approach and is associated with improved treatment outcome[113-115]. However, ongoing clinical trials, identification of novel biomarkers as well as further advances in high-throughput technologies will hopefully result in further development of therapeutic targets, treatment strategies and improved survival for these patients in the near future.

REFERENCES

1   **Mendelsohn J**. Personalizing oncology: perspectives and prospects. *J Clin Oncol* 2013; **31**: 1904-1911 [PMID: 23589547 DOI: 10.1200/JCO.2012.45.3605]
2   **Diaz Z**, Aguilar-Mahecha A, Paquet ER, Basik M, Orain M,

Camlioglu E, Constantin A, Benlimame N, Bachvarov D, Jannot G, Simard MJ, Chabot B, Gologan A, Klinck R, Gagnon-Kugler T, Lespérance B, Samson B, Kavan P, Alcindor T, Dalfen R, Lan C, Chabot C, Buchanan M, Przybytkowski E, Qureshi S, Rousseau C, Spatz A, Têtu B, Batist G. Next-generation biobanking of metastases to enable multidimensional molecular profiling in personalized medicine. *Mod Pathol* 2013; **26**: 1413-1424 [PMID: 23743930 DOI: 10.1038/modpathol.2013.81]

3   **Kim TM**, Lee SH, Chung YJ. Clinical applications of next-generation sequencing in colorectal cancers. *World J Gastroenterol* 2013; **19**: 6784-6793 [PMID: 24187453 DOI: 10.3748/wjg.v19.i40.6784]

4   **Pritzker KP**. Predictive and prognostic cancer biomarkers revisited. *Expert Rev Mol Diagn* 2015; **15**: 971-974 [PMID: 26134385 DOI: 10.1586/14737159.2015.1063421]

5   **Dienstmann R**, Dong F, Borger D, Dias-Santagata D, Ellisen LW, Le LP, Iafrate AJ. Standardized decision support in next generation sequencing reports of somatic cancer variants. *Mol Oncol* 2014; **8**: 859-873 [PMID: 24768039 DOI: 10.1016/j.molonc.2014.03.021]

6   **Zhang Y**. Epidemiology of esophageal cancer. *World J Gastroenterol* 2013; **19**: 5598-5606 [PMID: 24039351 DOI: 10.3748/wjg.v19.i34.5598]

7   **Findlay JM**, Middleton MR, Tomlinson I. A systematic review and meta-analysis of somatic and germline DNA sequence biomarkers of esophageal cancer survival, therapy response and stage. *Ann Oncol* 2015; **26**: 624-644 [PMID: 25214541 DOI: 10.1093/annonc/mdu449]

8   **Agrawal N**, Jiao Y, Bettegowda C, Hutfless SM, Wang Y, David S, Cheng Y, Twaddell WS, Latt NL, Shin EJ, Wang LD, Wang L, Yang W, Velculescu VE, Vogelstein B, Papadopoulos N, Kinzler KW, Meltzer SJ. Comparative genomic analysis of esophageal adenocarcinoma and squamous cell carcinoma. *Cancer Discov* 2012; **2**: 899-905 [PMID: 22877736 DOI: 10.1158/2159-8290.CD-12-0189]

9   **Lin DC**, Hao JJ, Nagata Y, Xu L, Shang L, Meng X, Sato Y, Okuno Y, Varela AM, Ding LW, Garg M, Liu LZ, Yang H, Yin D, Shi ZZ, Jiang YY, Gu WY, Gong T, Zhang Y, Xu X, Kalid O, Shacham S, Ogawa S, Wang MR, Koeffler HP. Genomic and molecular characterization of esophageal squamous cell carcinoma. *Nat Genet* 2014; **46**: 467-473 [PMID: 24686850 DOI: 10.1038/ng.2935]

10   **Song Y**, Li L, Ou Y, Gao Z, Li E, Li X, Zhang W, Wang J, Xu L, Zhou Y, Ma X, Liu L, Zhao Z, Huang X, Fan J, Dong L, Chen G, Ma L, Yang J, Chen L, He M, Li M, Zhuang X, Huang K, Qiu K, Yin G, Guo G, Feng Q, Chen P, Wu Z, Wu J, Ma L, Zhao J, Luo L, Fu M, Xu B, Chen B, Li Y, Tong T, Wang M, Liu Z, Lin D, Zhang X, Yang H, Wang J, Zhan Q. Identification of genomic alterations in oesophageal squamous cell cancer. *Nature* 2014; **509**: 91-95 [PMID: 24670651 DOI: 10.1038/nature13176]

11   **Dulak AM**, Stojanov P, Peng S, Lawrence MS, Fox C, Stewart C, Bandla S, Imamura Y, Schumacher SE, Shefler E, McKenna A, Carter SL, Cibulskis K, Sivachenko A, Saksena G, Voet D, Ramos AH, Auclair D, Thompson K, Sougnez C, Onofrio RC, Guiducci C, Beroukhim R, Zhou Z, Lin L, Lin J, Reddy R, Chang A, Landrenau R, Pennathur A, Ogino S, Luketich JD, Golub TR, Gabriel SB, Lander ES, Beer DG, Godfrey TE, Getz G, Bass AJ. Exome and whole-genome sequencing of esophageal adenocarcinoma identifies recurrent driver events and mutational complexity. *Nat Genet* 2013; **45**: 478-486 [PMID: 23525077 DOI: 10.1038/ng.2591]

12   **Forbes SA**, Beare D, Gunasekaran P, Leung K, Bindal N, Boutselakis H, Ding M, Bamford S, Cole C, Ward S, Kok CY, Jia M, De T, Teague JW, Stratton MR, McDermott U, Campbell PJ. COSMIC: exploring the world's knowledge of somatic mutations in human cancer. *Nucleic Acids Res* 2015; **43**: D805-D811 [PMID: 25355519 DOI: 10.1093/nar/gku1075]

13   **Wang K**, Johnson A, Ali SM, Klempner SJ, Bekaii-Saab T, Vacirca JL, Khaira D, Yelensky R, Chmielecki J, Elvin JA, Lipson D, Miller VA, Stephens PJ, Ross JS. Comprehensive Genomic Profiling of Advanced Esophageal Squamous Cell Carcinomas and Esophageal Adenocarcinomas Reveals Similarities and Differences. *Oncologist* 2015; **20**: 1132-1139 [PMID: 26336083 DOI: 10.1634/theoncologist.2015-0156]

14   **Fichter CD**, Timme S, Braun JA, Gudernatsch V, Schöpflin A, Bogatyreva L, Geddert H, Faller G, Klimstra D, Tang L, Hauschke D, Werner M, Lassmann S. EGFR, HER2 and HER3 dimerization patterns guide targeted inhibition in two histotypes of esophageal cancer. *Int J Cancer* 2014; **135**: 1517-1530 [PMID: 24510732 DOI: 10.1002/ijc.28771]

15   **Belkhiri A**, El-Rifai W. Advances in targeted therapies and new promising targets in esophageal cancer. *Oncotarget* 2015; **6**: 1348-1358 [PMID: 25593196]

16   **Yamamoto H**, Watanabe Y, Maehata T, Morita R, Yoshida Y, Oikawa R, Ishigooka S, Ozawa S, Matsuo Y, Hosoya K, Yamashita M, Taniguchi H, Nosho K, Suzuki H, Yasuda H, Shinomura Y, Itoh F. An updated review of gastric cancer in the next-generation sequencing era: insights from bench to bedside and vice versa. *World J Gastroenterol* 2014; **20**: 3927-3937 [PMID: 24744582 DOI: 10.3748/wjg.v20.i14.3927]

17   **Tan P**, Yeoh KG. Genetics and Molecular Pathogenesis of Gastric Adenocarcinoma. *Gastroenterology* 2015; **149**: 1153-1162.e3 [PMID: 26073375 DOI: 10.1053/j.gastro.2015.05.059]

18   **Nadauld LD**, Ford JM. Molecular profiling of gastric cancer: toward personalized cancer medicine. *J Clin Oncol* 2013; **31**: 838-839 [PMID: 23341521 DOI: 10.1200/JCO.2012.47.1714]

19   **Li X**, Wu WK, Xing R, Wong SH, Liu Y, Fang X, Zhang Y, Wang M, Wang J, Li L, Zhou Y, Tang S, Peng S, Qiu K, Chen L, Chen K, Yang H, Zhang W, Chan MT, Lu Y, Sung JJ, Yu J. Distinct Subtypes of Gastric Cancer Defined by Molecular Characterization Include Novel Mutational Signatures with Prognostic Capability. *Cancer Res* 2016; **76**: 1724-1732 [PMID: 26857262]

20   **Liang H**, Kim YH. Identifying molecular drivers of gastric cancer through next-generation sequencing. *Cancer Lett* 2013; **340**: 241-246 [PMID: 23178814 DOI: 10.1016/j.canlet.2012.11.029]

21   **Cancer Genome Atlas Research Network.** Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; **513**: 202-209 [PMID: 25079317 DOI: 10.1038/nature13480]

22   **Deng N**, Goh LK, Wang H, Das K, Tao J, Tan IB, Zhang S, Lee M, Wu J, Lim KH, Lei Z, Goh G, Lim QY, Tan AL, Sin Poh DY, Riahi S, Bell S, Shi MM, Linnartz R, Zhu F, Yeoh KG, Toh HC, Yong WP, Cheong HC, Rha SY, Boussioutas A, Grabsch H, Rozen S, Tan P. A comprehensive survey of genomic alterations in gastric cancer reveals systematic patterns of molecular exclusivity and co-occurrence among distinct therapeutic targets. *Gut* 2012; **61**: 673-684 [PMID: 22315472 DOI: 10.1136/gutjnl-2011-301839]

23   **Cui J**, Yin Y, Ma Q, Wang G, Olman V, Zhang Y, Chou WC, Hong CS, Zhang C, Cao S, Mao X, Li Y, Qin S, Zhao S, Jiang J, Hastings P, Li F, Xu Y. Comprehensive characterization of the genomic alterations in human gastric cancer. *Int J Cancer* 2015; **137**: 86-95 [PMID: 25422082 DOI: 10.1002/ijc.29352]

24   **Liu YJ**, Shen D, Yin X, Gavine P, Zhang T, Su X, Zhan P, Xu Y, Lv J, Qian J, Liu C, Sun Y, Qian Z, Zhang J, Gu Y, Ni X. HER2, MET and FGFR2 oncogenic driver alterations define distinct molecular segments for targeted therapies in gastric carcinoma. *Br J Cancer* 2014; **110**: 1169-1178 [PMID: 24518603 DOI: 10.1038/bjc.2014.61]

25   **O'Brien KM**, Orlow I, Antonescu CR, Ballman K, McCall L, DeMatteo R, Engel LS. Gastrointestinal stromal tumors, somatic mutations and candidate genetic risk variants. *PLoS One* 2013; **8**: e62119 [PMID: 23637977 DOI: 10.1371/journal.pone.0062119]

26   **Zhao X**, Yue C. Gastrointestinal stromal tumor. *J Gastrointest Oncol* 2012; **3**: 189-208 [PMID: 22943011 DOI: 10.3978/j.issn.2078-6891.2012.031]

27   **von Mehren M**, Randall RL, Benjamin RS, Boles S, Bui MM, Casper ES, Conrad EU, DeLaney TF, Ganjoo KN, George S, Gonzalez RJ, Heslin MJ, Kane JM, Mayerson J, McGarry SV, Meyer C, O'Donnell RJ, Pappo AS, Paz IB, Pfeifer JD, Riedel RF, Schuetze S, Schupak KD, Schwartz HS, Van Tine BA, Wayne JD, Bergman MA, Sundar H. Gastrointestinal stromal tumors, version 2.2014. *J Natl Compr Canc Netw* 2014; **12**: 853-862 [PMID: 24925196]

28   **Kee D**, Zalcberg JR. Current and emerging strategies for the management of imatinib-refractory advanced gastrointestinal

stromal tumors. *Ther Adv Med Oncol* 2012; **4**: 255-270 [PMID: 22942908 DOI: 10.1177/1758834012450935]

29 **Heinrich MC**, Maki RG, Corless CL, Antonescu CR, Harlow A, Griffith D, Town A, McKinley A, Ou WB, Fletcher JA, Fletcher CD, Huang X, Cohen DP, Baum CM, Demetri GD. Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor. *J Clin Oncol* 2008; **26**: 5352-5359 [PMID: 18955458 DOI: 10.1200/JCO.2007.15.746]

30 **Ferraro D**, Zalcberg J. Regorafenib in gastrointestinal stromal tumors: clinical evidence and place in therapy. *Ther Adv Med Oncol* 2014; **6**: 222-228 [PMID: 25342989 DOI: 10.1177/1758834014544892]

31 **Haggar FA**, Boushey RP. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. *Clin Colon Rectal Surg* 2009; **22**: 191-197 [PMID: 21037809 DOI: 10.1055/s-0029-1242458]

32 **Aran V**, Victorino AP, Thuler LC, Ferreira CG. Colorectal Cancer: Epidemiology, Disease Mechanisms and Interventions to Reduce Onset and Mortality. *Clin Colorectal Cancer* 2016; **15**: 195-203 [PMID: 26964802 DOI: 10.1016/j.clcc.2016.02.008]

33 **Heinemann V**, Stintzing S, Kirchner T, Boeck S, Jung A. Clinical relevance of EGFR- and KRAS-status in colorectal cancer patients treated with monoclonal antibodies directed against the EGFR. *Cancer Treat Rev* 2009; **35**: 262-271 [PMID: 19117687 DOI: 10.1016/j.ctrv.2008.11.005]

34 **De Roock W**, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, Kalogeras KT, Kotoula V, Papamichael D, Laurent-Puig P, Penault-Llorca F, Rougier P, Vincenzi B, Santini D, Tonini G, Cappuzzo F, Frattini M, Molinari F, Saletti P, De Dosso S, Martini M, Bardelli A, Siena S, Sartore-Bianchi A, Tabernero J, Macarulla T, Di Fiore F, Gangloff AO, Ciardiello F, Pfeiffer P, Qvortrup C, Hansen TP, Van Cutsem E, Piessevaux H, Lambrechts D, Delorenzi M, Tejpar S. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 2010; **11**: 753-762 [PMID: 20619739 DOI: 10.1016/S1470-2045(10)70130-3]

35 **Douillard JY**, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocáková I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Williams R, Rong A, Wiezorek J, Sidhu R, Patterson SD. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med* 2013; **369**: 1023-1034 [PMID: 24024839 DOI: 10.1056/NEJMoa1305275]

36 **Vogelstein B**, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Kinzler KW. Cancer genome landscapes. *Science* 2013; **339**: 1546-1558 [PMID: 23539594 DOI: 10.1126/science.1235122]

37 **Cancer Genome Atlas Network**. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; **487**: 330-337 [PMID: 22810696 DOI: 10.1038/nature11252]

38 **Brannon AR**, Vakiani E, Sylvester BE, Scott SN, McDermott G, Shah RH, Kania K, Viale A, Oschwald DM, Vacic V, Emde AK, Cercek A, Yaeger R, Kemeny NE, Saltz LB, Shia J, D'Angelica MI, Weiser MR, Solit DB, Berger MF. Comparative sequencing analysis reveals high genomic concordance between matched primary and metastatic colorectal cancer lesions. *Genome Biol* 2014; **15**: 454 [PMID: 25164765 DOI: 10.1186/s13059-014-0454-7]

39 **Yu J**, Wu WK, Li X, He J, Li XX, Ng SS, Yu C, Gao Z, Yang J, Li M, Wang Q, Liang Q, Pan Y, Tong JH, To KF, Wong N, Zhang N, Chen J, Lu Y, Lai PB, Chan FK, Li Y, Kung HF, Yang H, Wang J, Sung JJ. Novel recurrently mutated genes and a prognostic mutation signature in colorectal cancer. *Gut* 2015; **64**: 636-645 [PMID: 24951259 DOI: 10.1136/gutjnl-2013-30662]

40 **Dumur CI**. Available resources and challenges for the clinical annotation of somatic variations. *Cancer Cytopathol* 2014; **122**: 730-736 [PMID: 25111663 DOI: 10.1002/cncy.21471]

41 **Irahara N**, Baba Y, Nosho K, Shima K, Yan L, Dias-Santagata D, Iafrate AJ, Fuchs CS, Haigis KM, Ogino S. NRAS mutations are rare in colorectal cancer. *Diagn Mol Pathol* 2010; **19**: 157-163 [PMID: 20736745 DOI: 10.1097/PDM.0b013e3181c93fd1]

42 **Negru S**, Papadopoulou E, Apessos A, Stanculeanu DL, Ciuleanu E, Volovat C, Croitoru A, Kakolyris S, Aravantinos G, Ziras N, Athanasiadis E, Touroutoglou N, Pavlidis N, Kalofonos HP, Nasioulas G. KRAS, NRAS and BRAF mutations in Greek and Romanian patients with colorectal cancer: a cohort study. *BMJ Open* 2014; **4**: e004652 [PMID: 24859998 DOI: 10.1136/bmjopen-2013-004652]

43 **Di Nicolantonio F**, Martini M, Molinari F, Sartore-Bianchi A, Arena S, Saletti P, De Dosso S, Mazzucchelli L, Frattini M, Siena S, Bardelli A. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2008; **26**: 5705-5712 [PMID: 19001320 DOI: 10.1200/JCO.2008.18.0786]

44 **Davies H**, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA. Mutations of the BRAF gene in human cancer. *Nature* 2002; **417**: 949-954 [PMID: 12068308]

45 **Kalady MF**, Dejulius KL, Sanchez JA, Jarrar A, Liu X, Manilich E, Skacel M, Church JM. BRAF mutations in colorectal cancer are associated with distinct clinical characteristics and worse prognosis. *Dis Colon Rectum* 2012; **55**: 128-133 [PMID: 22228154 DOI: 10.1097/DCR.0b013e31823c08b3]

46 **Bardelli A**, Siena S. Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer. *J Clin Oncol* 2010; **28**: 1254-1261 [PMID: 20100961 DOI: 10.1200/JCO.2009.24.6116]

47 **Van Cutsem E**, Köhne CH, Láng I, Folprecht G, Nowacki MP, Cascinu S, Shchepotin I, Maurel J, Cunningham D, Tejpar S, Schlichting M, Zubel A, Celik I, Rougier P, Ciardiello F. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol* 2011; **29**: 2011-2019 [PMID: 21502544 DOI: 10.1200/JCO.2010.33.5091]

48 **Mao C**, Liao RY, Qiu LX, Wang XW, Ding H, Chen Q. BRAF V600E mutation and resistance to anti-EGFR monoclonal antibodies in patients with metastatic colorectal cancer: a meta-analysis. *Mol Biol Rep* 2011; **38**: 2219-2223 [PMID: 20857202 DOI: 10.1007/s11033-010-0351-4]

49 **Liu P**, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov* 2009; **8**: 627-644 [PMID: 19644473 DOI: 10.1038/nrd2926]

50 **Liao X**, Morikawa T, Lochhead P, Imamura Y, Kuchiba A, Yamauchi M, Nosho K, Qian ZR, Nishihara R, Meyerhardt JA, Fuchs CS, Ogino S. Prognostic role of PIK3CA mutation in colorectal cancer: cohort study and literature review. *Clin Cancer Res* 2012; **18**: 2257-2268 [PMID: 22357840 DOI: 10.1158/1078-0432.CCR-11-2410]

51 **Huang L**, Liu Z, Deng D, Tan A, Liao M, Mo Z, Yang X. Anti-epidermal growth factor receptor monoclonal antibody-based therapy for metastatic colorectal cancer: a meta-analysis of the effect of PIK3CA mutations in KRAS wild-type patients. *Arch Med Sci* 2014; **10**: 1-9 [PMID: 24701207 DOI: 10.5114/aoms.2014.40728]

52 **Sartore-Bianchi A**, Martini M, Molinari F, Veronese S, Nichelatti M, Artale S, Di Nicolantonio F, Saletti P, De Dosso S, Mazzucchelli L, Frattini M, Siena S, Bardelli A. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. *Cancer Res* 2009; **69**: 1851-1857 [PMID: 19223544 DOI: 10.1158/0008-5472.CAN-08-2466]

53 **Bronte G**, Silvestris N, Castiglia M, Galvano A, Passiglia F, Sortino G, Cicero G, Rolfo C, Peeters M, Bazan V, Fanale D, Giordano A, Russo A. New findings on primary and acquired resistance to anti-EGFR therapy in metastatic colorectal cancer: do all roads lead to RAS? *Oncotarget* 2015; **6**: 24780-24796 [PMID: 26318427 DOI: 10.18632/oncotarget.4959]

54 **Therkildsen C**, Bergmann TK, Henrichsen-Schnack T, Ladelund S,

Nilbert M. The predictive value of KRAS, NRAS, BRAF, PIK3CA and PTEN for anti-EGFR treatment in metastatic colorectal cancer: A systematic review and meta-analysis. *Acta Oncol* 2014; **53**: 852-864 [PMID: 24666267 DOI: 10.3109/0284186X.2014.895036]

55   **Moorcraft SY**, Smyth EC, Cunningham D. The role of personalized medicine in metastatic colorectal cancer: an evolving landscape. *Therap Adv Gastroenterol* 2013; **6**: 381-395 [PMID: 24003339 DOI: 10.1177/1756283X13491797]

56   **Liao X**, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, Imamura Y, Qian ZR, Baba Y, Shima K, Sun R, Nosho K, Meyerhardt JA, Giovannucci E, Fuchs CS, Chan AT, Ogino S. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. *N Engl J Med* 2012; **367**: 1596-1606 [PMID: 23094721 DOI: 10.1056/NEJMoa1207756]

57   **Khan KH**, Yap TA, Yan L, Cunningham D. Targeting the PI3K-AKT-mTOR signaling network in cancer. *Chin J Cancer* 2013; **32**: 253-265 [PMID: 23642907 DOI: 10.5732/cjc.013.10057]

58   **Wang XW**, Zhang YJ. Targeting mTOR network in colorectal cancer therapy. *World J Gastroenterol* 2014; **20**: 4178-4188 [PMID: 24764656 DOI: 10.3748/wjg.v20.i15.4178]

59   **Berg M**, Danielsen SA, Ahlquist T, Merok MA, Ågesen TH, Vatn MH, Mala T, Sjo OH, Bakka A, Moberg I, Fetveit T, Mathisen Ø, Husby A, Sandvik O, Nesbakken A, Thiis-Evensen E, Lothe RA. DNA sequence profiles of the colorectal cancer critical gene set KRAS-BRAF-PIK3CA-PTEN-TP53 related to age at disease onset. *PLoS One* 2010; **5**: e13978 [PMID: 21103049 DOI: 10.1371/journal.pone.0013978]

60   **De Roock W**, De Vriendt V, Normanno N, Ciardiello F, Tejpar S. KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. *Lancet Oncol* 2011; **12**: 594-603 [PMID: 21163703 DOI: 10.1016/S1470-2045(10)70209-6]

61   **Dillon LM**, Miller TW. Therapeutic targeting of cancers with loss of PTEN function. *Curr Drug Targets* 2014; **15**: 65-79 [PMID: 24387334]

62   **Liu J**, Hu J, Cheng L, Ren W, Yang M, Liu B, Xie L, Qian X. Biomarkers predicting resistance to epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer with wild-type KRAS. *Onco Targets Ther* 2016; **9**: 557-565 [PMID: 26869800 DOI: 10.2147/OTT.S86966]

63   **Janku F**, Hong DS, Fu S, Piha-Paul SA, Naing A, Falchook GS, Tsimberidou AM, Stepanek VM, Moulder SL, Lee JJ, Luthra R, Zinner RG, Broaddus RR, Wheler JJ, Kurzrock R. Assessing PIK3CA and PTEN in early-phase trials with PI3K/AKT/mTOR inhibitors. *Cell Rep* 2014; **6**: 377-387 [PMID: 24440717 DOI: 10.1016/j.celrep.2013.12.035]

64   **Carpten JD**, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, Hostetter G, Boguslawski S, Moses TY, Savage S, Uhlik M, Lin A, Du J, Qian YW, Zeckner DJ, Tucker-Kellogg G, Touchman J, Patel K, Mousses S, Bittner M, Schevitz R, Lai MH, Blanchard KL, Thomas JE. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature* 2007; **448**: 439-444 [PMID: 17611497]

65   **Davies BR**, Guan N, Logie A, Crafter C, Hanson L, Jacobs V, James N, Dudley P, Jacques K, Ladd B, D'Cruz CM, Zinda M, Lindemann J, Kodaira M, Tamura K, Jenkins EL. Tumors with AKT1E17K Mutations Are Rational Targets for Single Agent or Combination Therapy with AKT Inhibitors. *Mol Cancer Ther* 2015; **14**: 2441-2451 [PMID: 26351323 DOI: 10.1158/1535-7163.MCT-15-0230]

66   **Hechtman JF**, Sadowska J, Huse JT, Borsu L, Yaeger R, Shia J, Vakiani E, Ladanyi M, Arcila ME. AKT1 E17K in Colorectal Carcinoma Is Associated with BRAF V600E but Not MSI-H Status: A Clinicopathologic Comparison to PIK3CA Helical and Kinase Domain Mutants. *Mol Cancer Res* 2015; **13**: 1003-1008 [PMID: 25714871 DOI: 10.1158/1541-7786.MCR-15-0062-T]

67   **Edmonston TB**, Cuesta KH, Burkholder S, Barusevicius A, Rose D, Kovatich AJ, Boman B, Fry R, Fishel R, Palazzo JP. Colorectal carcinomas with high microsatellite instability: defining a distinct immunologic and molecular entity with respect to prognostic

markers. *Hum Pathol* 2000; **31**: 1506-1514 [PMID: 11150376]

68   **Starostik P**, Müller-Hermelink HK. Diagnosis of microsatellite instability-positive colorectal cancer. *Expert Rev Mol Diagn* 2001; **1**: 71-80 [PMID: 11901802]

69   **Boland CR**, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998; **58**: 5248-5257 [PMID: 9823339]

70   **Saridaki Z**, Souglakos J, Georgoulias V. Prognostic and predictive significance of MSI in stages II/III colon cancer. *World J Gastroenterol* 2014; **20**: 6809-6814 [PMID: 24944470 DOI: 10.3748/wjg.v20.i22.6809]

71   **Hampel H**, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, Clendenning M, Sotamaa K, Prior T, Westman JA, Panescu J, Fix D, Lockman J, LaJeunesse J, Comeras I, de la Chapelle A. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. *J Clin Oncol* 2008; **26**: 5783-5788 [PMID: 18809606 DOI: 10.1200/JCO.2008.17.5950]

72   **Weisenberger DJ**, Siegmund KD, Campan M, Young J, Long TI, Faasse MA, Kang GH, Widschwendter M, Weener D, Buchanan D, Koh H, Simms L, Barker M, Leggett B, Levine J, Kim M, French AJ, Thibodeau SN, Jass J, Haile R, Laird PW. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat Genet* 2006; **38**: 787-793 [PMID: 16804544]

73   **Parsons MT**, Buchanan DD, Thompson B, Young JP, Spurdle AB. Correlation of tumour BRAF mutations and MLH1 methylation with germline mismatch repair (MMR) gene mutation status: a literature review assessing utility of tumour features for MMR variant classification. *J Med Genet* 2012; **49**: 151-157 [PMID: 22368298 DOI: 10.1136/jmedgenet-2011-100714]

74   **Jacob S**, Aguado M, Fallik D, Praz F. The role of the DNA mismatch repair system in the cytotoxicity of the topoisomerase inhibitors camptothecin and etoposide to human colorectal cancer cells. *Cancer Res* 2001; **61**: 6555-6562 [PMID: 11522654]

75   **Kawakami H**, Zaanan A, Sinicrope FA. Microsatellite instability testing and its role in the management of colorectal cancer. *Curr Treat Options Oncol* 2015; **16**: 30 [PMID: 26031544 DOI: 10.1007/s11864-015-0348-2]

76   **Kawakami H**, Zaanan A, Sinicrope FA. Implications of mismatch repair-deficient status on management of early stage colorectal cancer. *J Gastrointest Oncol* 2015; **6**: 676-684 [PMID: 26697201 DOI: 10.3978/j.issn.2078-6891.2015.065]

77   **Le DT**, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med* 2015; **372**: 2509-2520 [PMID: 26028255 DOI: 10.1056/NEJMoa1500596]

78   **Goel G**. Evolving role of gene expression signatures as biomarkers in early-stage colon cancer. *J Gastrointest Cancer* 2014; **45**: 399-404 [PMID: 24989938 DOI: 10.1007/s12029-014-9634-7]

79   **O'Connell MJ**, Lavery I, Yothers G, Paik S, Clark-Langone KM, Lopatin M, Watson D, Baehner FL, Shak S, Baker J, Cowens JW, Wolmark N. Relationship between tumor gene expression and recurrence in four independent studies of patients with stage II/III colon cancer treated with surgery alone or surgery plus adjuvant fluorouracil plus leucovorin. *J Clin Oncol* 2010; **28**: 3937-3944 [PMID: 20679606 DOI: 10.1200/JCO.2010.28.9538]

80   **Gray RG**, Quirke P, Handley K, Lopatin M, Magill L, Baehner FL, Beaumont C, Clark-Langone KM, Yoshizawa CN, Lee M, Watson D, Shak S, Kerr DJ. Validation study of a quantitative multigene reverse transcriptase-polymerase chain reaction assay for assessment of recurrence risk in patients with stage II colon

cancer. *J Clin Oncol* 2011; **29**: 4611-4619 [PMID: 22067390 DOI: 10.1200/JCO.2010.32.8732]

81   **Venook AP**, Niedzwiecki D, Lopatin M, Ye X, Lee M, Friedman PN, Frankel W, Clark-Langone K, Millward C, Shak S, Goldberg RM, Mahmoud NN, Warren RS, Schilsky RL, Bertagnolli MM. Biologic determinants of tumor recurrence in stage II colon cancer: validation study of the 12-gene recurrence score in cancer and leukemia group B (CALGB) 9581. *J Clin Oncol* 2013; **31**: 1775-1781 [PMID: 23530100 DOI: 10.1200/JCO.2012.45.1096]

82   **Yothers G**, O'Connell MJ, Lee M, Lopatin M, Clark-Langone KM, Millward C, Paik S, Sharif S, Shak S, Wolmark N. Validation of the 12-gene colon cancer recurrence score in NSABP C-07 as a predictor of recurrence in patients with stage II and III colon cancer treated with fluorouracil and leucovorin (FU/LV) and FU/LV plus oxaliplatin. *J Clin Oncol* 2013; **31**: 4512-4519 [PMID: 24220557 DOI: 10.1200/JCO.2012.47.3116]

83   **Srivastava G**, Renfro LA, Behrens RJ, Lopatin M, Chao C, Soori GS, Dakhil SR, Mowat RB, Kuebler JP, Kim G, Mazurczak M, Lee M, Alberts SR. Prospective multicenter study of the impact of oncotype DX colon cancer assay results on treatment recommendations in stage II colon cancer patients. *Oncologist* 2014; **19**: 492-497 [PMID: 24710310 DOI: 10.1634/theoncologist.2013-0401]

84   **Francis G**, Stein S. Circulating Cell-Free Tumour DNA in the Management of Cancer. *Int J Mol Sci* 2015; **16**: 14122-14142 [PMID: 26101870 DOI: 10.3390/ijms160614122]

85   **Crowley E**, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. *Nat Rev Clin Oncol* 2013; **10**: 472-484 [PMID: 23836314 DOI: 10.1038/nrclinonc.2013.110]

86   **Bedard PL**, Hansen AR, Ratain MJ, Siu LL. Tumour heterogeneity in the clinic. *Nature* 2013; **501**: 355-364 [PMID: 24048068 DOI: 10.1038/nature12627]

87   **Diaz LA**, Williams RT, Wu J, Kinde I, Hecht JR, Berlin J, Allen B, Bozic I, Reiter JG, Nowak MA, Kinzler KW, Oliner KS, Vogelstein B. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature* 2012; **486**: 537-540 [PMID: 22722843 DOI: 10.1038/nature11219]

88   **Wong AL**, Lim JS, Sinha A, Gopinathan A, Lim R, Tan CS, Soh T, Venkatesh S, Titin C, Sapari NS, Lee SC, Yong WP, Tan DS, Pang B, Wang TT, Zee YK, Soong R, Trnkova Z, Lathia C, Thiery JP, Wilhelm S, Jeffers M, Goh BC. Tumour pharmacodynamics and circulating cell free DNA in patients with refractory colorectal carcinoma treated with regorafenib. *J Transl Med* 2015; **13**: 57 [PMID: 25889309 DOI: 10.1186/s12967-015-0405-4]

89   **Tabernero J**, Lenz HJ, Siena S, Sobrero A, Falcone A, Ychou M, Humblet Y, Bouché O, Mineur L, Barone C, Adenis A, Yoshino T, Goldberg RM, Sargent DJ, Wagner A, Laurent D, Teufel M, Jeffers M, Grothey A, Van Cutsem E. Analysis of circulating DNA and protein biomarkers to predict the clinical activity of regorafenib and assess prognosis in patients with metastatic colorectal cancer: a retrospective, exploratory analysis of the CORRECT trial. *Lancet Oncol* 2015; **16**: 937-948 [PMID: 26184520 DOI: 10.1016/S1470-2045(15)00138-2]

90   **Misale S**, Arena S, Lamba S, Siravegna G, Lallo A, Hobor S, Russo M, Buscarino M, Lazzari L, Sartore-Bianchi A, Bencardino K, Amatu A, Lauricella C, Valtorta E, Siena S, Di Nicolantonio F, Bardelli A. Blockade of EGFR and MEK intercepts heterogeneous mechanisms of acquired resistance to anti-EGFR therapies in colorectal cancer. *Sci Transl Med* 2014; **6**: 224ra26 [PMID: 24553387 DOI: 10.1126/scitranslmed.3007947]

91   **Spindler KL**, Pallisgaard N, Andersen RF, Jakobsen A. Changes in mutational status during third-line treatment for metastatic colorectal cancer--results of consecutive measurement of cell free DNA, KRAS and BRAF in the plasma. *Int J Cancer* 2014; **135**: 2215-2222 [PMID: 24659028 DOI: 10.1002/ijc.28863]

92   **Bardelli A**, Corso S, Bertotti A, Hobor S, Valtorta E, Siravegna G, Sartore-Bianchi A, Scala E, Cassingena A, Zecchin D, Apicella M, Migliardi G, Galimi F, Lauricella C, Zanon C, Perera T, Veronese S, Corti G, Amatu A, Gambacorta M, Diaz LA, Sausen M, Velculescu VE, Comoglio P, Trusolino L, Di Nicolantonio F, Giordano S, Siena S. Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. *Cancer Discov* 2013; **3**: 658-673 [PMID: 23729478 DOI: 10.1158/2159-8290.CD-12-0558]

93   **Kwak EL**, Ahronian LG, Siravegna G, Mussolin B, Godfrey JT, Clark JW, Blaszkowsky LS, Ryan DP, Lennerz JK, Iafrate AJ, Bardelli A, Hong TS, Corcoran RB. Molecular Heterogeneity and Receptor Coamplification Drive Resistance to Targeted Therapy in MET-Amplified Esophagogastric Cancer. *Cancer Discov* 2015; **5**: 1271-1281 [PMID: 26432108 DOI: 10.1158/2159-8290.CD-15-0748]

94   **Russo M**, Siravegna G, Blaszkowsky LS, Corti G, Crisafulli G, Ahronian LG, Mussolin B, Kwak EL, Buscarino M, Lazzari L, Valtorta E, Truini M, Jessop NA, Robinson HE, Hong TS, Mino-Kenudson M, Di Nicolantonio F, Thabet A, Sartore-Bianchi A, Siena S, Iafrate AJ, Bardelli A, Corcoran RB. Tumor Heterogeneity and Lesion-Specific Response to Targeted Therapy in Colorectal Cancer. *Cancer Discov* 2016; **6**: 147-153 [PMID: 26644315 DOI: 10.1158/2159-8290.CD-15-1283]

95   **Shoda K**, Ichikawa D, Fujita Y, Masuda K, Hiramoto H, Hamada J, Arita T, Konishi H, Komatsu S, Shiozaki A, Kakihara N, Okamoto K, Taniguchi H, Imoto I, Otsuji E. Monitoring the HER2 copy number status in circulating tumor DNA by droplet digital PCR in patients with gastric cancer. *Gastric Cancer* 2016 Feb 13; Epub ahead of print [PMID: 26874951]

96   **Takegawa N**, Yonesaka K, Sakai K, Ueda H, Watanabe S, Nonagase Y, Okuno T, Takeda M, Maenishi O, Tsurutani J, Satoh T, Okamoto I, Nishio K, Tamura T, Nakagawa K. HER2 genomic amplification in circulating tumor DNA from patients with cetuximab-resistant colorectal cancer. *Oncotarget* 2016; **7**: 3453-3460 [PMID: 26657506 DOI: 10.18632/oncotarget.6498]

97   **Montagut C**, Dalmases A, Bellosillo B, Crespo M, Pairet S, Iglesias M, Salido M, Gallen M, Marsters S, Tsai SP, Minoche A, Seshagiri S, Serrano S, Himmelbauer H, Bellmunt J, Rovira A, Settleman J, Bosch F, Albanell J. Identification of a mutation in the extracellular domain of the Epidermal Growth Factor Receptor conferring cetuximab resistance in colorectal cancer. *Nat Med* 2012; **18**: 221-223 [PMID: 22270724 DOI: 10.1038/nm.2609]

98   **Morelli MP**, Overman MJ, Dasari A, Kazmi SM, Mazard T, Vilar E, Morris VK, Lee MS, Herron D, Eng C, Morris J, Kee BK, Janku F, Deaton FL, Garrett C, Maru D, Diehl F, Angenendt P, Kopetz S. Characterizing the patterns of clonal selection in circulating tumor DNA from patients with colorectal cancer refractory to anti-EGFR treatment. *Ann Oncol* 2015; **26**: 731-736 [PMID: 25628445 DOI: 10.1093/annonc/mdv005]

99   **Maier J**, Lange T, Kerle I, Specht K, Bruegel M, Wickenhauser C, Jost P, Niederwieser D, Peschel C, Duyster J, von Bubnoff N. Detection of mutant free circulating tumor DNA in the plasma of patients with gastrointestinal stromal tumor harboring activating mutations of CKIT or PDGFRA. *Clin Cancer Res* 2013; **19**: 4854-4867 [PMID: 23833305 DOI: 10.1158/1078-0432.CCR-13-0765]

100   **Kang G**, Bae BN, Sohn BS, Pyo JS, Kang GH, Kim KM. Detection of KIT and PDGFRA mutations in the plasma of patients with gastrointestinal stromal tumor. *Target Oncol* 2015; **10**: 597-601 [PMID: 25735500 DOI: 10.1007/s11523-015-0361-1]

101   **Meldrum C**, Doyle MA, Tothill RW. Next-generation sequencing for cancer diagnostics: a practical perspective. *Clin Biochem Rev* 2011; **32**: 177-195 [PMID: 22147957]

102   **Tucker T**, Marra M, Friedman JM. Massively parallel sequencing: the next big thing in genetic medicine. *Am J Hum Genet* 2009; **85**: 142-154 [PMID: 19679224 DOI: 10.1016/j.ajhg.2009.06.022]

103   **Liu L**, Li Y, Li S, Hu N, He Y, Pong R, Lin D, Lu L, Law M. Comparison of next-generation sequencing systems. *J Biomed Biotechnol* 2012; **2012**: 251364 [PMID: 22829749 DOI: 10.1155/2012/251364]

104   **Gagan J**, Van Allen EM. Next-generation sequencing to guide cancer therapy. *Genome Med* 2015; **7**: 80 [PMID: 26221189 DOI: 10.1186/s13073-015-0203-x]

105   **Chin L**, Hahn WC, Getz G, Meyerson M. Making sense of cancer genomic data. *Genes Dev* 2011; **25**: 534-555 [PMID: 21406553

DOI: 10.1101/gad.2017311]

106   **Wu TJ**, Schriml LM, Chen QR, Colbert M, Crichton DJ, Finney R, Hu Y, Kibbe WA, Kincaid H, Meerzaman D, Mitraka E, Pan Y, Smith KM, Srivastava S, Ward S, Yan C, Mazumder R. Generating a focused view of disease ontology cancer terms for pan-cancer data integration and analysis. *Database* (Oxford) 2015; **2015**: bav032 [PMID: 25841438 DOI: 10.1093/database/bav032]

107   **Loman NJ**, Misra RV, Dallman TJ, Constantinidou C, Gharbia SE, Wain J, Pallen MJ. Performance comparison of benchtop high-throughput sequencing platforms. *Nat Biotechnol* 2012; **30**: 434-439 [PMID: 22522955 DOI: 10.1038/nbt.2198]

108   **Chen H**, Luthra R, Goswami RS, Singh RR, Roy-Chowdhuri S. Analysis of Pre-Analytic Factors Affecting the Success of Clinical Next-Generation Sequencing of Solid Organ Malignancies. *Cancers* (Basel) 2015; **7**: 1699-1715 [PMID: 26343728 DOI: 10.3390/cancers7030859]

109   **Zhang L**, Chen L, Sah S, Latham GJ, Patel R, Song Q, Koeppen H, Tam R, Schleifman E, Mashhedi H, Chalasani S, Fu L, Sumiyoshi T, Raja R, Forrest W, Hampton GM, Lackner MR, Hegde P, Jia S. Profiling cancer gene mutations in clinical formalin-fixed, paraffin-embedded colorectal tumor specimens using targeted next-generation sequencing. *Oncologist* 2014; **19**: 336-343 [PMID: 24664487 DOI: 10.1634/theoncologist.2013-0180]

110   **Gargis AS**, Kalman L, Berry MW, Bick DP, Dimmock DP, Hambuch T, Lu F, Lyon E, Voelkerding KV, Zehnbauer BA, Agarwala R, Bennett SF, Chen B, Chin EL, Compton JG, Das S, Farkas DH, Ferber MJ, Funke BH, Furtado MR, Ganova-Raeva LM, Geigenmüller U, Gunselman SJ, Hegde MR, Johnson PL, Kasarskis A, Kulkarni S, Lenk T, Liu CS, Manion M, Manolio TA, Mardis ER, Merker JD, Rajeevan MS, Reese MG, Rehm HL, Simen BB, Yeakley JM, Zook JM, Lubin IM. Assuring the quality of next-generation sequencing in clinical laboratory practice. *Nat Biotechnol* 2012; **30**: 1033-1036 [PMID: 23138292 DOI: 10.1038/nbt.2403]

111   **Rehm HL**, Bale SJ, Bayrak-Toydemir P, Berg JS, Brown KK, Deignan JL, Friez MJ, Funke BH, Hegde MR, Lyon E. ACMG clinical laboratory standards for next-generation sequencing. *Genet Med* 2013; **15**: 733-747 [PMID: 23887774 DOI: 10.1038/gim.2013.92]

112   **de Leng WW**, Gadellaa-van Hooijdonk CG, Barendregt-Smouter FA, Koudijs MJ, Nijman I, Hinrichs JW, Cuppen E, van Lieshout S, Loberg RD, de Jonge M, Voest EE, de Weger RA, Steeghs N, Langenberg MH, Sleijfer S, Willems SM, Lolkema MP. Targeted Next Generation Sequencing as a Reliable Diagnostic Assay for the Detection of Somatic Mutations in Tumours Using Minimal DNA Amounts from Formalin Fixed Paraffin Embedded Material. *PLoS One* 2016; **11**: e0149405 [PMID: 26919633 DOI: 10.1371/journal.pone.0149405]

113   **Jardim DL**, Schwaederle M, Wei C, Lee JJ, Hong DS, Eggermont AM, Schilsky RL, Mendelsohn J, Lazar V, Kurzrock R. Impact of a Biomarker-Based Strategy on Oncology Drug Development: A Meta-analysis of Clinical Trials Leading to FDA Approval. *J Natl Cancer Inst* 2015; **107**: [PMID: 26378224 DOI: 10.1093/jnci/djv253]

114   **Schwaederle M**, Zhao M, Lee JJ, Lazar V, Leyland-Jones B, Schilsky RL, Mendelsohn J, Kurzrock R. Association of Biomarker-Based Treatment Strategies With Response Rates and Progression-Free Survival in Refractory Malignant Neoplasms: A Meta-analysis. *JAMA Oncol* 2016 Jun 6; Epub ahead of print [PMID: 27273579 DOI: 10.1001/jamaoncol.2016.2129]

115   **Schwaederle M**, Zhao M, Lee JJ, Eggermont AM, Schilsky RL, Mendelsohn J, Lazar V, Kurzrock R. Impact of Precision Medicine in Diverse Cancers: A Meta-Analysis of Phase II Clinical Trials. *J Clin Oncol* 2015; **33**: 3817-3825 [PMID: 26304871 DOI: 10.1200/JCO.2015.61.5997]