Review Article

Biomarkers of Colorectal Cancer

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Abstract

Colorectal cancer (CRC) which affects over one million individuals annually [1] has recently seen an increased interest in identifying biomarkers and new treatment strategies. This has resulted in a significant rise in the median overall survival of CRC from 6 to 24 months [2]. Standard treatment for CRC has progressed from 5-flurururacil monotherapy to combination chemotherapy (5-flurouracil and irinotecan and/or oxaliplatin) and more recently to biological agents targeted at angiogenesis and the epidermal growth factor receptor (EGFR). The monoclonal EGFR antibodies, cetuximab and panitumumab have been shown to be effective in 10-15% of metastatic CRC patients. Patients who do not benefit from cetuximab or panitumumab have a *K-RAS* mutation at codon12. However patients with a *K-RAS* mutation at codon 13 (i.e. G13D) do respond positively to EGFR antibody therapy. Therefore it is recommended that the mutational status of *K-RAS* should be assessed in every CRC patient prior to initiating treatment with EGFR antibodies [2]. Recent advances in multiplex genotyping technologies and high-throughput genomic profiling by next-generation technologies make possible the rapid and comprehensive analysis of the cancer genome of individual patients even from very little tumor biopsy material. Predictive biomarkers using molecular diagnostics are currently in use in clinical practice of CRC oncotherapy and are successfully being used to evaluate benefits that can be achieved through molecularly targeted biomarker therapies (tyrosine kinase inhibitors). Prognostic biomarkers are useful in identifying somatic germ line mutations in CRC. This review discusses the current status of research on biomarkers for CRC and summarizes data on emerging therapeutic targets and emerging validated predictive biomarkers for CRC [3-5].

ABBREVIATION

AKT: Cell Signaling Pathway also called Protein Kinase B (PKB) and Phosphoin ositide 3-Kinase (PI3K); APC: Adenomatous Polyposis Gene; B-*Raf V600E*: B-*Raf* is a 766-Amin o Acid. Regulated Signal Transduction Serine/Threonin e-Specific Protein Kinase; BRAF V600E: is a Determinant Of Sensitivity To Proteasome Inhibitors; BRAF: A Human Gene that Makes A Protein B-Raf; CC: Colon Cancer; CEA: Carcino Embryonic Antigen; CIMP: Cpg Island Methylator Phenotype; CIN : Chromosomal In stability; CTC: Circulating Tumor Cells; COX-2: Cycloxygenase 2; CRC: Colorectal Cancer ; DKK4: Dickkopf Homolog 4 Protein; DPD: Dihydro Pyrimidine Dehydrogenase ; DNA: Deoxyribo Nucleic Acid; EGFR : Epidermal Growth Factor Receptor; EGTM: European Group on Tumor Markers; FAP: Familial Adenomatous Polyposis; FIT-Based FOBT: Fecal Immunochemical Test (FIT) Based Fecal Occult Blood Test; GIST: Gastroin testinal Stroma; GOJ: Gastro-Esophageal Junction; HNPCC: Hereditary Nonpolyposis Colorectal Cancer (Lynch Syndrome); K-RAS: The KRAS gene belongs to a class of genes Known as oncogenes. When mutated, oncogenes have the potential to cause normal cells to become cancerous. The KRAS gene is in the Ras family of oncogenes. This also includes two other genes HRAS and NRAS. The proteins produced from these three genes are Gtpases. These proteins play important roles in cell division, cell differentiation and the self-destruction of cells (Apoptosis); K-Ras G_{13}D: The G_{13}D mutation results in an amino acid substitution at position 13 in KRAS, from a glycine (G) to an aspartic acid; LOE: Level Of Evidence; MCC: Metastatic Colorectal Cancer ; MAPK: Mitogen-Activated Protein Kinases: Origin ally Called ERK: Extracellular Signal-Regulated Kinases); MEK: Mitogen Activated Protein Kinase; Mi RNA: Micro RNA; MLH1: Mutl Homolog 1; MPH2: Muts Protein Homolog 2; MSI/DMMR: Microsatellite In stability/ Deficient Mismatch Repair; NRAS: Neuroplastoma RAS Viral (V-Ras) Oncogene Homologue ; PTEN : Phosphate and Tensin Homologue Deleted on Chromosome 10; PIK3CA: Phosphatidylin ositol-4:5-Biphosphate 3-Kinase: Catalytic Subunit Alpha; RC: Rectal Cancer; RNA: Ribonucleic Acid; SOR: Strength of Recommendation; TFAP2E: Gene Encoding Transcription Factor AP-E Epsilon. This gene is associated with chemoresistance in colorectar cancer; UGT 1A1*:* Uridine Glucoronyl Transferase; VEGF: Vascular Endothelial Growth Factor.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common malignant neoplasm in both men and women with an estimated 96,830 cases of colon cancer and 50,310 deaths from colon cancer predicted for 2014 [1]. Colorectal cancer affects the inner wall of the colon (CC) and the rectum (RC) located within 12 cm or less from the anal verge [6]. CRC is characterized by late clinical manifestation and relatively rapid progression of the disease, both features that are primarily responsible for increased morbidity and mortality in patients suffering from this disease [7]. There is new evidence that epigenetic slicing of genes may be an early event in the adenoma carcinoma sequence [8,9]. If CRC is diagnosed early enough, it can be considered to be preventable. This makes it critical that inexpensive, highly sensitive, specific and non-invasive biomarkers be developed [7]. The last decade has seen significant advances in the development of screening, predictive, prognostic and risk assessment biomarkers in CRC that play a critical role in understanding molecular and cellular mechanisms that drive tumor initiation, maintenance and progression (Tables 1-3). Clinical molecular diagnostics and biomarker discoveries are uncovering the complex mechanisms underlying CRC. These discoveries have fueled the development of novel drug targets and new treatment strategies. The standard of care of care for patients with advanced-stage cancers CRC has shifted away from an empirical treatment strategy based on the clinical-pathological profile to one where a biomarker driven treatment algorithm based on the molecular profile of the tumor is now being used. Predictive (diagnostic) biomarkers are helpful in matching targeted therapies with patients and in preventing toxicity of standard (systemic) therapies. Predictive biomarkers using molecular diagnostics that are currently in use in clinical practice of personalized oncotherapy for the treatment of colon cancer are being used successfully to evaluate benefits that can be achieved through molecularly targeted biomarker therapies (anti- EGFR tyrosine kinase inhibitors). Prognostic biomarkers identify somatic germ line mutations, changes in DNA methylation, elevated levels of microRNA (miRNA), circulating tumor cells (CTC) in blood, carcino-embryonic antigen (CEA) and microsatellite instability (MSI) and deficient mismatch repair (DMMR) (Table 2).

EGFR GENE EXPRESSION

Epidermal growth factor receptor (EGFR) is a transmembrane ligand-induced receptor that elicits its effect by activating pathways that promote tumor proliferation, invasion, migration and neovascularization [2], approximately 70% of human colorectal cancers express EGFR protein. The anti-EGFR monoclonal antibodies such as cetuximab (a chimeric IgG1 antibody) and panitumumab (a humanized IgG2 antibody) competitively inhibit EGFR by preventing its binding to endogenous ligands. These antibodies have been found to be effective in all lines of metastatic CRC treatment. It has been found the cetuximab has prolonged survival – particularly when given in combination with chemotherapy. The common side effect of anti-EGFR therapy (rash) has been used as a predictor of a positive response [18]. In some cases mutations in signaling pathways downstream of EGFR (such as mutations in KRAS codons 12 and 13) may render CRC tumors unresponsive to anti-

EGFR blockage treatment [2]. For this reason, testing of KRAS exon 2 mutations is now used for selecting patients for anti-EGFR treatment.

GENETICS OF COLORECTAL CANCER

In general CRCs do not have a recognizable inherited cause [10]. Sporadically occurring CRC cases, which account for 75% - 80% of all CRCs in the population, are the result of complex interactions between susceptibility genes and environmental facts. A family history of the disease, increasing age, male gender, obesity, alcohol consumption, diet rich in total fat and cigarette smoking are among CRC risk factors, while dietary regimens rich in fiber, folate and other vitamins, are associated with a decreased risk [10]. Only 5% of all CRC cases are due to inherited conditions i.e. due to inheritance of a highly mutated gene, predisposing an individual to the development of the disease such as the following CRC genetic syndromes: Familial Adenomatous Polyposis (FAP), attenuated FAP, MUTYH-associated polyposis, hereditary nonpolyposis colorectal cancer (HNPCC: Lynch syndrome) and hamartomatous polyposis syndromes [10-12]. Fewer than 10% of patients have an inherited predisposition to colon cancer. Familial adenomatous polyposis patients inherit a mutated copy of the adenomatous polyposis gene (APC) and hereditary non-polyposis colon cancer is caused by inherited mutations of mismatched repair genes (MLH1, MSH2, PMS2 and MSH6). Certain frequent gene alterations have recently been discovered [13] by the use of conventional as well as the very advanced techniques such as next-generation sequencing (NGS) based studies of CRC genomes. These have helped identify of some unique mutational spectrums and novel targets of genomic alterations associated with CRC along with their biological and clinical significance [14].

EPIGENETICS OF COLORECTAL CANCER

CRC, like other malignancies, is now recognized to be a heterogeneous disease involving genetic and epigenetic alterations that transform normal colonic epithelium into cancer [10]. Epigenetics are mechanisms able to modify gene expression levels without necessarily altering the DNA sequence. Epigenetics are environmentally-mediated, frequent, powerful and widespread heritable changes in gene expression that are not attributable to permanent changes in DNA sequence itself but are sufficiently powerful to regulate the dynamics of gene expression [15,16].

The primary processes responsible for epigenetic regulation include DNA methylation, histone modifications and posttranscriptional gene regulation through non-coding RNAs (microRNAs [miRNAs], long non-coding RNAs, small nucleolar RNAs etc) [17]. Of these epigenetic mechanisms, DNA methylation is associated with repression of gene expression and represents a more chemically and biologically stable source of molecular diagnostic information than RNA or most proteins. Recent studies on large cohorts of CRC patients have revealed a molecular heterogeneity of CRC with different subtypes that are characterized by distinct genetic, cytogenetic and epigenetic alterations [10]. Epigenetic biomarkers are increasing becoming recognized as valuable diagnostic and even as prognostic biomarkers. In the future, a better knowledge of the crosstalk

between genetic and epigenetic modifications during CRC carcinogenesis will permit the design of preventable strategies in healthy individuals, detect early disease and personalize treatment for each patient [11].

Over the past seven years, several studies have specifically looked for circulating miRNAs in patients with CRC with preliminary results that are encouraging [6,7]. So far three miRNAs (miR-21-5p, miR-29-3p, miR-148-3p) have been examined in several studies [6]. However, the clinical applicability of the use of these miRNAs as prognostic biomarkers of CRC has yet to be established.

BIOMARKERS OF COLORECTAL CANCER IN THE PIPELINE AND EMERGING THERAPEUTIC TARGETS FOR THE TREATMENT OF CRC

The European Group on Tumor Markers (EGTM) has published a list of emerging therapeutic targets for CRC. Cetuximab and panitumumab are monoclonal antibodies that bind to the extracellular domain of epidermal growth factor receptor (EGFR), thereby inhibiting downstream signaling and resulting in decreased cell proliferation and migration [3].

Apart from these monoclonal antibodies, there are no other validated predictive markers currently available for the treatment of CRC. Other emerging therapeutic targets for the treatment of CRC are: bevacizumab and aflibercept targeting VEGF; regorafenib targeting multikinases (VEGFR1, VEGFR2, VEGFR3, PDGFRbeta, Tie-2, FGFR1, RET and BRAF); vemutafenib and dabrafenib targeting mutant BRAF; selutmetinib and pimasertib targeting MEK; evorilimus tareting mTOR and LY294002 and GDC0941 are targeting P13K [3]. In addition the EGTM recently published updated and new guidelines on the use of biomarkers for CRC, gastrointestinal stromal (GISTs), and gastric and gastro-esophageal junction (GOJ) cancers. (Table 2) is the summary of the 2014 guidelines for biomarkers for CRC which has been modified from Duffy et al 2014 [3] (Table 3). Summarizes the predictive and prognostic biomarkers for colorectal cancer (CRC) that are in the pipeline. Several of these biomarkers for CRC are considered to be "emerging biomarkers" or CRC biomarkers in the pipeline: 1) K-*ras G13D* gene mutation; 2) VEGF; 3) micro RNAs; 4) Microsatellite instability (MSI); 5) Cycloxygenase 2 (COX–2); 6) CpG Island Methylator Phenotype (CIMP); 7) Chromosomal instability (CIN); 8) B-raf murine sarcoma viral oncogene homolog B (B-*raf*), *V600E* gene mutation.

K-RAS G13D gene mutation

Ras genes are among the most frequently activated oncogenes. K-*ras* is found in adenocarcinomas that transduces extracellular signals from the EGFR to the nucleus. K-*ras* is the only predictive biomaker established for anti-EGFR monoclonal antibody in colorectal cancer. Approximately 40% of colon cancers are positive for mutations in K-*ras* in codons 12,13,61 of colorectal cancer and are resistant to anti-EGFR monoclonal antibodies (cetuximab and panitumumab). A diagnostic kit was recently approved to determine whether or not patients with advanced colorectal cancer have a wild K-*ras* gene that could indicate whether they would respond to cetuximab or pantiumumab [18]. The current standard for patients with all types of K-*ras* gene mutations is not to treat with anti-EGFR monoclonal antibodies

(cetuximab and panitumumab). In addition to K-*ras* gene mutation the specific genotype will be important in developing future therapy for these tumors [18].

VEGF

Angiogenesis plays an important role in progression of colorectal carcinoma (CRC). Evidence from preclinical and clinical studies indicates that vascular endothelial growth factor (VEGF) is the predominant angiogenic factor in CRC. Indeed, VEGF is expressed in approximately 50% of CRCs, with minimal to no expression in normal colonic mucosa and adenomas. Quantification of VEGF-1 expression seems to provide valuable prognostic information in CRC, particularly in selecting those patients at high risk for disease progression who are likely to benefit from adjuvant therapy. Emerging biomarkers for CRC are bevacizumab and aflibercept targeting VEGF; regorafenib targeting multikinases (VEGFR1, VEGFR2, VEGFR3, PDGFRbeta, Tie-2, FGFR1, RET and BRAF) [23].

Micro RNAs

Micro RNAs are small non-coding RNAs mediating the regulation of gene expression in carcinogenesis. Over the past seven years, several studies have specifically looked for circulating miRNAs in patients with CRC with preliminary results that are encouraging [6,7]. So far three miRNAs (miR-21-5p, miR-29-3p, and miR-148-3p) have been examined in several studies [6]. Several well-characterized cancer-related genes are being investigates as novel putative miRNA targets [23].

Microsatellite instability (MSI)

Microsatellite instability (MSI) is a hypermutable phenotype caused by the loss of DNA mismatch repair activity. MSI is detected in about 15% of all colorectal cancers; 3% are of these are associated with Lynch syndrome and the other 12% are caused by sporadic, acquired hypermethylation of the promoter of the *MLH1* gene, which occurs in tumors with the CpG island methylator phenotype. Colorectal tumors with MSI have distinctive features, including a tendency to arise in the proximal colon, lymphocytic infiltrate, and a poorly differentiated, mucinous or signet ring appearance. They have a slightly better prognosis than colorectal tumors without MSI and do not have the same response to chemotherapeutics. Discovery of MSI in colorectal tumors has increased awareness of the diversity of colorectal cancers and implications for specialized management of patients [23].

Cycloxygenase -2 (COX-2) Inhibitor

COX-2 mediates the inflammatory effects of COX activity, and is induced by a wide spectrum of growth factors and proinflammatory cytokines, and is over expressed in numerous premalignant and malignant lesions, including CRC. Treatment with the selective COX-2 inhibitor celecoxib has shown promising results in the prevention of CRC. Numerous studies show that this COX-2 selective inhibitor is a potent suppressor of colon polyps both in animal models for familial adenomatous polyposis and in patients with this condition [23].

CpG Island Methylator Phenotype (CIMP)

Genetic instability is an important engine of molecular

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diversity of colon cancer the clinical implications of multiple pathways to colon cancer affect all aspects of colon cancer – from screening to therapy. Interventions to prevent colon cancer may have a preferential effect on one disease type but not on the others, an effect that might lead to false-negative trials (by dilution). From a screening standpoint, efforts are under way to use molecular markers in stool or serum for the detection of colon cancer. The biggest effect of the multiple pathways to colon cancer model relates to therapy. Despite decades of research, there continues to be uniformity in thinking of, and in treating colon cancer. The different colon cancers have vastly different prognoses, ranging from favorable (MSI, BRAF unmutated) to very poor (CIMP, no MSI). There is increasing recognition of different responsiveness of MSI cases to 5-fluorouracil (which likely also extends to CIMP cases), and clinical trials will prospectively test treating these patients differently. Most recently, cases with *KRAS* mutations (most of which belong to the CIMP group) have been shown to have a very low response rate to adding cetuximab to chemotherapy [23].

Chromosomal instability (CIN)

The acquisition of genomic instability is a crucial feature in tumor development and there are at least 3 distinct pathways in colorectal cancer pathogenesis: the chromosomal instability (CIN), microsatellite instability, and CpG island methylator phenotype pathways. Most cases of colorectal cancer arise through the CIN pathway, which is characterized by widespread imbalances in chromosome number (aneuploidy) and loss of heterozygosity.

It can result from defects in chromosomal segregation, telomere stability, and the DNA damage response, although the full complement of genes underlying CIN remains incompletely described. Coupled with the karyotypic abnormalities observed in CIN tumors are the accumulation of a characteristic set of mutations in specific tumor suppressor genes and oncogenes that activate pathways critical for colorectal cancer initiation and progression. Whether CIN creates the appropriate milieu for the accumulation of these mutations or vice versa remains a provocative and unanswered question [23].

B-*Raf V600E* gene mutation

B-raf murine sarcoma viral oncogene homolog B (B-*raf*), *V600E* gene mutation. This is considered to be an emerging biomarker of negative response to K-*ras.* Currently B-*raf* is a mutation found in 10=20% of colorectal cancers and is considered to be a prognostic biomarker for poor prognosis in patients with first-line (initial therapy) colon cancer therapies [18] (Tables 1,2).

DIHYDRO PYRIMIDINE DEHYDROGENASE (DPD) DEFICIENCY

This is the major fluorouracil (a chemotherapeutic agent for colorectal cancers), catabolizing enzyme encoded by *DPYD,* Approximately 3-5% of individuals in the general population have partial enzyme deficiency. The predictive and prognostic value of DPD deficiency has yet to be established [18].

Table 1: Classification of Biomarkers of colotrectal cancer based on their biological roles (screening, predictive, prognostic and risk assessment) in clinical practice (*modified from Kalia 2014* [3,4]).

Biological Role	Biomarker	Abnormality	Mechanism of Action	Therapy If applicable
Screening	DNA panel	genetic mutations		
Screening	stool DNA profile	genetic mutations		
Screening	septin 9	genetic mutation		
Predictive	anti-EGF tyrosine kinase inhibitors (antibodies)	genetic mutation (15-20%)	Blocks EGFR signaling. KRAS mutations are associated with a lack of benefit from anti-EGFR antibodies. Wild-type KRAS + anti-EGFR antibodies+ chemotherapy enhance patient outcomes Testing of mutational status of KRAS is now standard practice in patient identification in metastatic CRC.	Cetuximab Panitumumab Imatinib
Predictive	KRAS	Genetic mutation exon 2 (4-15%).Used for patient selection with wild-type KRAS for treatment with anti-EGFR antibodies	A proto-oncogene involved in cellular response to extracellular stimuli. KRAS mutation involves A structural activation of downstream signaling pathways i.e. MAPK and P13K/AKT.	
Predictive	BRAF	Genetic V600E mutation in 10-20% of CRC	A signature of BRAF/KRAS a possible predictive factor for the response to EGFR inhibitors.	
Predictive	NRAS (neuroblastoma RAS viral (v-ras) oncgene)	Genetic mutation	The encoded protein, which has intrinsic GTPase activity, is activated by a guanine nucleotide-exchange factor and inactivated by a GTPase activating protein.	
Predictive	PTEN (Phosphatase and tensin) homolog protein	Genetic mutation. Encoded by the PTEN gene	*PTEN* acts as a tumor suppressor gene through the action of its phosphatase protein product. This phosphatase is involved in the regulation of the cell cycle, preventing cells from growing and dividing too rapidly.	

Predictive	PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha).	Like other kinases, PI3K adds a cluster of oxygen and phosphorus atoms (a phosphate group) to other proteins through a process called phosphorylation.	PI3K signaling is important for many cell activities, including cell growth and division (proliferation), movement (migration) of cells, production of new proteins, transport of materials within cells, and cell survival.	
Prognostic	BRAF	Genetic V600 mutation in 10-20% of CRC	Associated with sporadic MSI positive tumors through its relationship with CIMP-high. Mutated BRAF is one of the most powerful prognostic markers in CRC.	
Prognostic	VEGF	proangiogenic factor	Involved in cell proliferation, migration, and vascular permeability. Increase in VEGF expression is associated with poor prognosis, low response to preoperative radiotherapy and greater likelihood of Recurrence.	
Prognostic	TIMP metallopeptidase inhibitor	protein coding	involved in the degradation of the extracellular matrix	
Predictive of response to chemotherapy	Dihydropyrimidine dehydrogenase (DPD)	gene expression of DPD	enzyme involved in pyrimidine degradation. Involved in the degradation of chemotherapeutic drugs:5-fluorouracil and Tegafururacil.	5-fluorouracil and Tegafururacil
Response to chemotherapy	AP-2 epsilon (*TFAP2E*)	Genomic and epigenetic alterations gene encoding transcription factor	The gene encoding homolog 4 protein (*DKK4*) is a potential downstream target of *TFAP2E* and has been implicated in chemotherapy resistance.	
Predictive of toxicity of irinotecan	UGT 1A1	Genetic mutation	Responsible for irinotecan glucuronidation	Irinotecan
Risk assessment	MLH1/MSH2	Genetic mutation	Member of the MMR (mismatch repair) gene family. Increases the risk of tumor formation. example Lynch Syndrome	

B-*raf* V600E: B-*raf* is a 766-amino acid, regulated signal transduction serine/threonine-specific protein kinase, BRAF V600E is a determinant of sensitivity to proteasome inhibitors, CIMP.

DPH: DihydroPyrimidine Dehydrogenase; EGFR: Epidermal Growth Factor Receptor; K-*ras* $G_{13}D$: The G13D mutation results in an amino acid substitution at position 13 in KRAS, from a glycine (G) to an aspartic acid; MAPK: Mitogen-Activated Protein Kinase; mCRC: metastatic Colorectal Cancer; MLH1/ MLH2, MSI ; NRAS: Neuroplastoma RAS Viral (v-ras) Oncogene Homologue; P13K/AKT: Phosphoinositide-3-Kinase/V-Akt Murine Thymoma Viral Oncogen; PTEN: Phosphate and Tensin Homologue Deleted on Chromosome 10; PIK3CA: Phosphatidylinositol-4,5-Biphosphate 3-Kinase, Catalytic Subunit Alpha; VEGF: Vascular Endothelial Growth Factor; UGT 1A1: Uridine Glucoronyl Transferase.

UGT1A1 I GENOTYPING

This is the enzyme that metabolizes irinotecan (a colorectal cancer chemotherapeutic agent) to its active metabolite SN-38. The prognostic value of *UGT1A1* has not yet been established [18].

SUMMARY AND CONCLUSIONS

The central goal of biomarker-based personalized colon cancer therapy is to make treatment decisions based on tumor genotypes and genetic profiles. Matching targeted therapies against specific genetic aberrations is an important step for personalized colon cancer therapy. Such an approach holds promise in ultimately improving measurable clinical outcomes: response rates, survival and safety [19-21]. A new molecular classification of colon cancers has evolved based on chromosomal aberrations, gene mutations and signaling pathway activation that underlie biologically unique tumors that now need to be managed clinically in several different ways. Early clinical application of these technologies has made possible the rapid and comprehensive molecular annotation of an individual's cancer. This facilitates the identification of actionable and/or novel drug targets and treatment options, as well as the characterization of underlying pathogenic mechanisms [22]. Understanding molecular carcinogenesis will continue to shape the approach taken towards CRC. Patients will be treated with preferentially targeted substances based on specific molecular profiles found in individual tumor tissues [22]. miRNA's are becoming more relevant in the diagnosis, sub-classification, and prognosis and as biomarkers of CRC and the use of genomic DNA could be used to monitor residual disease. In the future, mutational testing and the development of serological biomarkers will enable us to stratify patients for targeted therapies. This new paradigm in drug development and clinical care has created both opportunities and challenges. Numerous multiplex genotyping platforms are being evaluated for actionable hotspot oncogene mutations or gene amplifications or rearrangements are being evaluated with promising results that are progressing towards use in CRC [22,23]. The biggest challenge in personalized colorectal cancer treatment involving translating cancer genomics is to understand how these aberrations are related to the progression of the CRC over time. In spite of this knowledge gap, these recent advances

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Table 2: Biomarkers recommended by the European Group for Tumor Markers (EGTM) for use in colorectal cancer (CRC) (Modified from Duffy et al 2013).

Biomarker	Use	Level of Evidence (LOE)	Strength of Recommendation (SOR)
FIT-based FOBT	Screening	I	A
MSI/DMMR	Prescreen for Lynch syndrome	I	A
K-RAS	Predicting response/ Resistance to Anti-EGFR antibodies	I	A
CEA	Postoperative Surveillance	I	A
CEA	Monitoring therapy In advanced disease	III	A
CEA	Prognosis, especially in stage II	III	A
MSI/DMMR	Prognosis especially in stage ii disease	I	A

Mod Modified from Duffy 2013
CEA: Carcino Embryonic Antigen; EGFR: Epidermal Growth Factor Receptor; FIT-based FOBT: Fecal Immunochemical Test (FIT) based Fecal Occult Blood Test; *K-RAS:* The KRAS gene belongs to a class of genes known as oncogenes. When mutated, oncogenes have the potential to cause normal cells to become cancerous. The KRAS gene is in the Ras family of oncogenes, which also includes two other genes, HRAS and NRAS. The proteins produced from these three genes are GTPases. These proteins play important roles in cell division, cell differentiation, and the self-destruction of cells (apoptosis). LOE: Level of Evidence; MSI/dMMR: Microsatellite Instability/Deficient Mismatch Repair; SOR: Strength of Recommandation.

Table 3: Predictive and Prognostic Biomarkers for Colorectal Cancer in the Pipeline.

CRC Biomarkers tunder development	Type of Biomarker	Mechanism of action	Role in CRC
KRAS (Kirsten Rat Sarcoma) G13D gene mutation	Predictive	proto-oncogene which encodes a GTP-ase involved incellular response to extracellular stimuli	Indicator of a Better response to EGFR inhibitors with standard chemotherapy
VEGF (vascular endothelial growth factor) Gene expression	Predictive	pro-angiogenic factor	Linked to the aggressiveness of CRC
micro RNAs:	Predictive and Prognostic	short 18-25 nucleotide (non-coding) single-stranded RNA sequences Involved inregulating gene expression. Down regulation of the following: miR-451, miR-624, miR-29c, miR-126, miR-129, miR-133,	Indicators of poor Prognosis in CRC
Microsatellite instability (MSI)	Prognostic	Point mutations in defect mismatch repair system of DNA (15%)	Indicator of poor prognosis Correlate with other significant mutations e.g. KRAS and BRAF
Cycloxygenase 2 (COX-2)	Prognostic	COX-2 inhibitors associated with a lower risk of CRC Risk also strongly correlated with BRAF and VEGF	Associated with worse Outcomes in CRC
CpG Island Methylator Phenotype (CIMP)	Prognostic	Methylation of CpG islands of suppressor promoters	Indicator of poor prognosis Correlate with other significant Mutations eg. KRAS and BRAF
Chromosomal instability (CIN)	Prognostic	Abnormal chromosome Complement or number	Indicator of poor prognosis correlates with other significant mutations eg. KRAS and BRAF
v-raf murine sarcoma viral oncogene homolog B (BRAF)	Prognostic	V600E mutation A serine-threonine protein kinase	Indicator of poor prognosis

Based on data from Sideris and Papagrigoriadis, 2014
BRAF – V600 mutation; v-raf murine sarcoma viral oncogene homolog; V 600E mutation;
CIMP; CpG Island- Methylator Phenotype; CIN: Chromosomal Instability; CRC: Colorectal Cancer; COX 2: Cycloxygenase 2; EGFR: Epidermal Growth Factor; KRAS: Kirsten Rat Sarcoma Viral Oncogene Homoogue; MSI: Microsatellite Instability; Mir: Micro RNAs; VEGF: Vascular Enothelial Growth Factor.

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in identifying biomarkers using genomic technologies continue to make great strides - developments which hold enormous promise for advancing colorectal cancer treatment.

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