

Colorectal Cancer: Molecular Classification And Clinical Application

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Abstract— Genetic -depth study showed the perplexity of molecular heterogeneity of colorectal cancer (CRC). Though various therapies exist, we do not have the proper way to choose the right treatment for each patient, personalized treatment strategies are in demand. For CRC, a broad molecular classification is still missing. We wish to apply the molecular techniques to improve the outcome. Our intention in this review is to summarize the molecular classification of CRC and their reflection on management.

Index Terms—Colorectal cancer, molecular classification, colon cancer subtypes.

I. INTRODUCTION

Colorectal cancer (CRC) is one of the major causes of morbidity and mortality, more than 1.2 million patients are diagnosed every year, and more than 600,000 die from the disease, more common in old age men; median age at diagnosis is about 70 years in developed countries [1]. CRC is not one disease, although with the same stage of CRC, the response to treatment may be different; could be explained by the molecular heterogeneity, either genetic or epigenetic. In spite of great interest, the molecular classification of CRC has not achieved widespread clinical application, and has not been approved by many oncology centers. Better understanding of molecular classification will help us to assimilate the process of carcinogenesis and may contribute to create novel and more effective therapy [2]. In this review, we summarize the molecular pathways and classification of CRC and the impact on patients.

II. EVOLUTION OF MOLECULAR CLASSIFICATION OF CRC REVIEW STAGE

The transformation from normal epithelium to carcinoma is associated with many molecular events. In CRC carcinogenesis, there are three major distinguished molecular pathways have been involved: chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP) [3].

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The first and most common (70%), the CIN pathway, is characterized by frequent loss of heterozygosity (LOH) in chromosomes, alternation in the main oncogenes (e.g., KRAS, NRAS, BRAF, PIK3) and tumor suppressor genes (e.g., APC, TP53, and PTEN). Key pathways include Wnt/ β -catenin, transforming growth factor beta (TGF- β), epidermal growth factor receptor (EGFR, HER1), downstream mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI3K) signaling activation [4].

The second pathway is the MSI, occurs in 15% of CRC and caused by inactivation of DNA mismatch repair genes (MMR). The presence of MSI represents phenotypic evidence that MMR is not functioning normally (d MMR). CRC with MSI has a clear molecular origin and a specific clinicopathological phenotype; associated with poor differentiated tissue, high mucinogens, tumor infiltrating lymphocytes, right-sided location, and enriched with BRAF mutations [5].

In addition to CIN and MSI, a third epigenetic instability pathway (found in approximately 15%–20% of CRC) was explained by Toyota et al, CpG island methylation phenotype (CIMP), characterized by vast hypermethylation of promoter CpG island sites, resulting in the inactivation of several tumor suppressor genes or other tumor-related genes [6]. The accurate description of CIMP has not been equal among studies; actually there are different classifications for CIMP tumors. Table 1 illustrates some of these classifications.

The three molecular pathways are not mutually exclusive, so they may be exhibited in the same CRC patient. Actually, there are many proposals for molecular classification of CRC but without complete agreement. According to clinical, morphological, and molecular parameters, Jeremy Jass proposed a model included five subgroups [7]. The Cancer Genome Atlas (TCGA) subcategorized CRC depending on mutation rate into hypermutated and nonhypermutated group [8].

Another classification system was published by Domingo et al, dividing CRC into 7 groups [9]. Based on genetic and clinicopathological characteristics, De Sousa E et al. identified 3 different subtypes of CRC [10]. In other study done by Sadanandam et al, 5 different types of CRC were identified based on gene expression profiles [11]. Noticeable thing that, BRAF mutations were found in sessile serrated adenoma (SSA) either in early hyperplastic polyps or in the advanced dysplastic form, reflecting its role in neoplastic progression [12].

Importantly, over the last decade, it has been documented that other pathways are implicated in the pathogenesis of CRC, as microRNA (miRNA) and inflammatory pathways. The early reports about miRNA denoting its low expression

level in cancer, anticipated that they were tumor suppressors. In CRC it is not the truth, more microRNAs have been elevated [13].

Table (2) illustrates some of molecular classifications CRC.

Trezić et al, has established the relation between inflammation and CRC either sporadic or heritable types. Recently, the role of immune mediators has been clarified in CRC carcinogenesis, from tumor initiation till metastasis. The proposed mechanisms may include production of many angiogenic factors hand in hand with DNA damage [14].

III. CLINICAL APPLICATION

The rapid evolution in the identification of molecular basis of CRC has led to discovery of novel drugs and molecular diagnostics markers. The clinical use of monoclonal antibodies (mAbs); cetuximab/ panitumumab, targeting EGFR is an excellent example; moreover, nearly all patients whom initially respond inevitably become refractory [15].

The mechanisms of resistance to EGFR mAbs in CRCs include, range from genetic alterations in the pathway to amplification of receptor tyrosine kinases (TKR). The mechanisms may be assigned as primary resistance as genetic alterations in the RAS-RAF-MEK pathway, HER2 amplification, and MET amplification or acquired resistance such as the EGFR mutation S492R [16].

Moreover, Cetuximab have antibody-dependent cell-mediated cytotoxicity (ADCC) which depends on the interaction between antibody Fc portion and Fc receptors (FcγRs) in immune cells. Bibeau et al, [17] demonstrated that combined FcγRIIa and FcγRIIIa polymorphisms are prognostic factors for progression-free survival in mCRC patients treated with cetuximab plus irinotecan, which is corresponding to the study done by Kjersem et al, [18].

Whereas polymorphisms are clinically linked to mutated-KRAS mCRC, an important role of ADCC in cetuximab efficacy is assumed. However, due to retrospective studies most always are criticized as the completeness of data often is suboptimal and depends totally on medical documentation, ancillary studies to larger prospective clinical trials are needed to assess the impact of Fcγ R polymorphisms on cetuximab efficiency.

Also, pioneering work demonstrated that antibodies containing engineered bisected increase ADCC amplification. This therapeutic mechanism is likely to be important when simple interference with receptor/ligand interactions fails as a therapeutic strategy [19], and this strategy is undergoing clinical validation.

Diaz et al, detected the KRAS mutations not only in tumor biopsies but also in circulating tumor DNA (ctDNA) in patients with acquired resistance [20]. This may change the method of genetic alteration detection from multiple tumor biopsies to just drawing a tube of blood, what is called liquid biopsies or ctDNA, which is a specific cancer biomarker that can be detected, measured, and tracked. Preliminary data suggests ctDNA is detectable at diagnosis in the majority of patients with non-metastatic CRC. The potential for ctDNA as a CRC screening tool, and as a prognostic marker for early stage, should be further explored [21].

Surprising, not all mutations in KRAS gene have the same biologic behavior. In an analysis, use of cetuximab was associated with longer overall and progression-free survival among patients with chemotherapy-refractory CRC with p.G13D-mutated tumors than with other KRAS-mutated tumors [22, 23].

Considerable preclinical data have shown that the combination of ERBB tyrosine kinase inhibitors and anti-EGFR mAbs leads to markedly higher antitumor activity than the administration of single agents, especially in KRAS wild-type and Quadruple-negative (KRAS/NRAS/BRAF/PIK3CA wild-type) tumors [24- 26].

There are a lot of studies accused HER3 signaling pathways activation and compensatory PI3K pathway activation as a cause of anti-EGFR therapeutics failure. Preclinical cancer models have indicated that patritumab (fully human anti-HER3 monoclonal antibody) demonstrates antitumor activity when used alone or with anti-EGFR inhibitors by binding to the extracellular domain of HER3 and promoting the internalization and degradation of the receptor [27].

With limited clinical benefit from the use of BRAF inhibitors as single agents in BRAF V600E-mutated CRC, the clinical trials tend to investigate BRAF inhibitors in combinations; either with anti-EGFR mAbs or with third agent (MEK or PI3K pathway inhibitors) [28, 29].

Programmed death 1 (PD1) and its ligand (PD-L1), are highly expressed in a variety of cancers and hence the role in cancer immune therapy is well established [30]. Gatalica, et al, presented a poster in 2014 ASCO Annual Meeting, concluded, the expression of PD-1 and PD-L1+ cancer cells are more frequent in MSI-H than in MSS CRC, which are rare in general CRC population, subsequently the use of anti-PD-1 mAb perhaps hold new hope for treatment [31]. We summarized some clinical trials in CRC in Table 3.

In a retrospective multicenter study including 782 patients with CRC, post surgery, revealed that the combination of PIK3CA mutations with MSS were associated with good prognosis and postulated that they may not require adjuvant chemotherapy [32].

Reimers et al, observed that, the benefits of the adjuvant aspirin were belonged to CRC patients with COX-2-positive or PIK3CA mutation-negative [33].

MSI is an important piece of information to consider when deciding adjuvant chemotherapy in stage II CRC. Data from the PETACC3 trial suggested that MSI-H tumors have a decreased like hood of metastasis, which is considered as prognostic marker for favorable outcome [34-36]. A retrospective study involving long term follow up of patients with stage II and III CRC have found that patients with stage II MSI-H tumors not only did not derive any benefit from 5-FU adjuvant therapy, but they actually fared worse if they were treated [35]. Similar results were showed by Sargent et al, [36]. In contrast to these finding, a study done by Hutchins et al, from QUASAR study showed that although MSI-H was a prognostic, it did not predict benefit from or detrimental effect on chemotherapy [37]. This corresponding to study done by Bertagnolli et al, on patients in the CALGB and 89803 trials [38].

Although the overall result of National Surgical Adjuvant Breast and Bowel Project protocol C-08 was negative, the data suggest that there may be a subset of CRC patients may get clinical benefit from the addition of bevacizumab to adjuvant chemotherapy, but it needs independent validation in other clinical trials [39].

MicroRNAs are endogenous posttranscriptional modulators that control the expression of the target genes and play an important role in the development and progression of many malignancies, including CRC. Toyama et al, revealed that Serum miR-21 is a promising biomarker for the early detection and prognosis of CRC [40]. Several studies have shown an association between elevated levels of miR21 and the down regulation of tumor suppressor genes, this has led to miR21 being considered a promising therapeutic target for treating CRC [41].

Table 1: CRC classification based on CIMP status

Reference	Method of evaluation	Types	Clinical notes
Weisenberger <i>et al.</i> (42)	Methy light technology	- CIMP-positive -CIMP-negative	-A great correlation of CIMP cancers with BRAF mutations.
Shen <i>et al.</i> (43)	Methy light technology	-CIMP-positive (1,2) -CIMP-negative	-CIMP1 tumors are often MSI tumors (80%), and have BRAF mutations (53%), -CIMP2 tumors have KRAS mutations (92%), rarely are MSI or have BRAF or TP53 mutations
Ogino <i>et al.</i> (44)	Quantified DNA methylation in five CIMP-specific gene promoters [CACNA1G, CDKN2A (p16), CRABP1, MLH1, and NEUROG1]	-CIMP-low (CRC with 1/5 to 3/5 methylated promoters) -CIMP-high (4/5 or 5/5 methylated promoters) -CIMP-0 (0/5 methylated promoters)	-CIMP-low CRC is associated with male sex and KRAS mutations.
Barault <i>et al.</i> (45)	Quantified DNA methylation in five CIMP-specific gene promoters (hMLH1, p16, MINT1, MINT2, and MINT31)	-No CIMP -CIMP-low -CIMP-high	- Methylation is an independent prognostic factor in MSS CRC
Ang <i>et al.</i> (46)	GoldenGate [®] methylation array	-CIMP-low -CIMP-mid -CIMP-high	-In comparison to CIMP-L tumors, CIMP-H tumors were more often located in the proximal colon and showed more frequent mutation of <i>KRAS</i> and <i>B</i>

			RAF
Yagi <i>et al.</i> (47)	Methylated DNA immunoprecipitation on-on-chip	-Low methylation epigenotypes -Intermediate methylation epigenotypes -High methylation epigenotypes	-Three methylation epigenotypes exist in colorectal cancer, and suitable classification markers have been developed. Intermediate-methylation epigenotype with KRAS-mutation (+) correlated with worse prognosis.

Table 2: Some General Molecular CRC Classification

Item	Description			
Jeremy <i>et al.</i>	-Group 1 chromosomally stable (CS), MLH1 methylated, MSI-H, BRAF mutated, CIMP high. Group 2 CS, partially MLH1 methylated, microsatellite stable (MSS), MSI-L, BRAF mutated, CIMP high. Group 3 CIN, MGMT methylated, MSS/MSI-L, KRAS mutated, CIMP low. Group 4 CIN, MSS/MSI-L, CIMP negative. Group 5 CS, MSI-H, CIMP negative, BRAF wild type.			
The Cancer Genome Atlas Somatic events	The hypermutated Mismatch-repair genes	Nonhypermutated	TP53 mutations, which characterize CIN	
BRAF mutations	DNA repair gene alteration:	More gene copy number		
KRAS and PIK3CA mutations	About 50% of cases	Less than 5% of cases.		
	Very uncommon	Common		
Domingo <i>et al.</i>	Group 1 MSI-H and/or BRAF mutated Group 2 CIN and/or TP53 mutated with KRAS and PIK3CA wild type. Group 3 CIN, KRAS and/or PIK3CA mutated; TP53 wild type. Group 4 CS, KRAS and/or PIK3CA mutated; TP53 wild type. Group 5 NRAS mutated. Group 6 no mutations. Group 7 other.			
De Soma <i>et al.</i>	CCS1	CCS2	CCS3	CCS4
DFS	Good	Intermediate		
Gene signature	Poor	Inflammatory		
Precursor	Epithelial			
Wnt signaling	Mesenchymal	Unknown		
	SSAs			
	adenoma			
	High	Low		Low
Sadanandam <i>et al.</i>	Stem-like Inflammatory type	Transit amplifying	Goblet cell	Enterocyte
		RC-TA CS-TA		
DFS	Poor	Poor	Good	Intermediate
Gene signature	Intermediate			
Colon crypt top base	Mesenchymal	Heterogeneous	Goblet cell	Enterocyte cell
Wnt signaling	Increased cytokines	with TA cell		
	Stem cell			
	Base	TopBase	Top	Top
	Unknown			
	High	High/Low	Low	Low
	Unknown			

CRC, colorectal cancer; CS, chromosomally stable; CIN; microsatellite instability; MSI, microsatellite instability; MSS, microsatellite stability; CIMP, CpG island methylator phenotype; CCS, colon cancer subtypes; DFS, disease-free survival; SSA, sessile serrated adenoma; TA, transit-amplifying; CR-TA, cetuximab-resistant TA; CS-TA, cetuximab-sensitive TA; DFS, disease-free survival;

Table 3: Selected Clinical Trials In Advanced CRC Based On Biological Hallmarks.

NCT ID	Trial description	Study type and design	Trial phase	Last updated
NCT02227667	<ul style="list-style-type: none"> To Evaluate the Efficacy of MEDI4736 in Immunological subsets of advanced colorectal cancer. Drug: MEDI4736; Durvalumab; Fc optimized monoclonal antibody directed against programmed cell death-1. 	Interventional	Phase II	April 23, 2015
NCT02442414	<ul style="list-style-type: none"> To determine the maximum tolerated dose of KBP-5209 as a single agent for patients with advanced colorectal cancer after failure of standard chemotherapy. Drug: KBP-5209; Plectinib; is a second-generation, irreversible pan-EGFR tyrosine kinase inhibitor 	Interventional	Phase I	May 8, 2015
NCT01376505	<ul style="list-style-type: none"> To evaluate the side effects and best dose of vaccine therapy in treating patients with advanced colorectal cancer. Biological: HER-2 vaccine. 	Interventional	Phase I	May 18, 2015
NCT01304602	<ul style="list-style-type: none"> To Evaluate the Efficacy of BKM120 in patients with advanced colorectal cancer after failure or intolerance of at least one line of therapy. Drug: BKM120; Buparlisib; PI3K inhibitor 	Interventional	Phase I	March 31, 2015
NCT01776307	<ul style="list-style-type: none"> To Evaluate the Efficacy of BBI608 in combination with cetuximab, panitumumab or capecitabine in patients with advanced colorectal cancer after failure of at least 2 regimens containing 5-Fluorouracil, oxaliplatin, or irinotecan Drug: BBI608; is a cancer stem cell inhibitor. 	Interventional	Phase II	August 28, 2015
NCT02512172	<ul style="list-style-type: none"> To evaluate the safety and effectiveness of the combination of intravenous ramipril and/or 5-azacitidine with IV MK-3475 in patients with Microsatellite stable. Drug: MK-3475; antibody directed against programmed cell death-1 	Interventional	Phase I	July 27, 2015
NCT02537418	<ul style="list-style-type: none"> To evaluate the side effects and best dose of durvalumab alone or combined with tremelimumab in advanced colorectal cancer after failure of standard chemotherapy. Drug: Durvalumab; antibody directed against programmed cell death-1 Tremelimumab; antibody activate the immune system through blocking the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) 	Interventional	Phase I	August 31, 2015
NCT01802320	<ul style="list-style-type: none"> For patients advanced colorectal cancer after failure or intolerance of at least one line of therapy, with KRAS Wild Type, enriched for PTEN Loss and PIK3CA Mutation. Drug: MK2206; Akt Inhibitor 	Interventional	Phase II	August 24, 2015
NCT01937715	<ul style="list-style-type: none"> To evaluate safety and Efficacy of PF-05212384 with FOLFIRI regimen in ACRCA*. Drug: PF-05212384; pan-class I isoform PI3K/mTOR inhibitor. 	Interventional	Phase II	August 18, 2015
NCT01253628	<ul style="list-style-type: none"> To evaluate the safety and efficacy of a cetuximab and PX-866 combination treatment in advanced colorectal cancer. Drug: PX-866; PI3K inhibitor 	Interventional	Phase I	June 16, 2015

IV. CONCLUSION

Although the introduction of epigenetic modifications and methylated genes may help in further identification, in fact, at this time, there is no sharp difference between many molecular classifications based on histological or clinical features. There is a growing need to universal disease classification system that engages clinical and molecular features to personalize the treatment and assess if there is a relationship between these subtypes and survival endpoints hoping to reduce disease burden in the future.

CONFLICT OF INTEREST

The authors certify that there is no actual or potential conflict of interest in relation to this article.

REFERENCES

- [1] Brenner H, Kloor M, Pox CP. Colorectal cancer. *Lancet*. 2014; 383:1490–502.
- [2] Yamauchi M, Lochhead P, Morikawa T, Huttenhower C, Chan AT, Giovannucci E, et al. Colorectal cancer: a tale of two sides or a continuum? *Gut*. 2012; 61:794–797.
- [3] Yamagishi H, Kuroda H, Imai Y, Hiraishi H. Molecular pathogenesis of sporadic colorectal cancers *Chin J Cancer*. 2016; 6:35-4.
- [4] Pino MS, Chung DC. The chromosomal instability pathway in colon cancer. *Gastroenterology*. 2010; 138:2059-2072.
- [5] Vilar E, Taberero J. Molecular dissection of microsatellite instable colorectal cancer. *Cancer Discov*. 2013; 3:502-511.
- [6] Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci U S A*. 1999; 96:8681–8686.
- [7] Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. *Histopathology*. 2007; 50:113–30.
- [8] Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature*. 2012; 487:330-337.
- [9] Domingo E, Ramamoorthy R, Oukrif D, Rosmarin D, Presz M, Wang H, et al. Use of multivariate analysis to suggest a new molecular classification of colorectal cancer. *J Pathol*. 2013; 229:441–448.
- [10] De Sousa E, Wang X, Jansen M, Fessler E, Trinh A, de Rooij LP, et al. Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. *Nat Med* 2013; 19:614-618.
- [11] Sadanandam A, Lyssiotis CA, Homicicko K, Collisson EA, Gibb WJ, Wullschlegel S, et al. A colorectal cancer classification system that associates cellular phenotype and responses to therapy. *Nat Med*. 2013; 19:619-625.
- [12] Spring KJ, Zhao ZZ, Karamatic R, Walsh MD, Whitehall VL, Pike T, et al. High prevalence of sessile serrated adenomas with BRAF mutations: a prospective study of patients undergoing colonoscopy. *Gastroenterology* 2006; 131:1400–7.
- [13] Colussi D, Brandi G, Bazzoli F, Ricciardiello L. Molecular pathways involved in colorectal cancer: implications for disease behavior and prevention. *Int J Mol Sci* 2013; 14:16365–85.
- [14] Terzic J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. *Gastroenterology* 2010; 138:2101–14.
- [15] Dienstmann R, Vilar E, Taberero J. Molecular predictors of response to chemotherapy in colorectal cancer. *Cancer J*. 2011; 17:114-126.
- [16] Leto SM, and Trusolino L. "Primary and acquired resistance to EGFR targeted therapies in colorectal cancer: impact on future treatment strategies". *J Mol Med* 2014 Jul 92:709-22
- [17] Bibeau F, Lopez-Crapez E, Di Fiore F, Thezenas S, Ychou M, Blanchard F, et al., Impact of Fc{gamma}RIIIa Fc{gamma}RIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan. *J Clin Oncol* 1:1122-9.
- [18] Kjersem JB, Skovlund E, Ikdahl T, Guren T, Kersten C, Dalsgaard AM, et al. FCGR2A and FCGR3A polymorphisms and clinical outcome in metastatic colorectal cancer patients treated with first-line 5-fluorouracil/folinic acid and oxaliplatin +/- cetuximab. *BMC Cancer*. 2014, 14:340.
- [19] Gerdes CA, Nicolini VG, Herter S, van Puijenbroek E, Lang S, Roemmele M, Moessner E, Freytag O, Friess T, Ries CH, Bossenmaier B, Mueller HJ, Umana P (2013) GA201 (RG7160): a novel, humanized, glycoengineered anti-EGFR antibody with enhanced ADCC and superior in vivo efficacy compared with cetuximab. *Clin Cancer Res* 19: 1126–1138
- [20] Diaz LA Jr, Williams RT, Wu J, Kinde I, Hecht JR, Berlin J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature*. 2012; 486:537-540.
- [21] Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Isaac K, et al. Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies. *Sci Transl Med*. 2014; 19: 6: 224ra24.
- [22] Tejpar S, Celik I, Schlichting M, Sartorius U, Bokemeyer C, Van Cutsem E. Association of KRAS G13D tumor mutations with outcome in patients with metastatic colorectal cancer treated with first-line

- chemotherapy with or without cetuximab. *J Clin Oncol.* 2012; 30:3570-3577.
- [23] De Roock W, Jonker DJ, Di Nicolantonio F, Sartore-Bianchi A, Tu D, Siena S, et al. Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. *JAMA.* 2010; 304:1812-1820.
- [24] Weickhardt AJ, Price TJ, Chong G, GebSKI V, Pavlakis N, Johns TG, et al. Dual targeting of the epidermal growth factor receptor using the combination of cetuximab and erlotinib: preclinical evaluation and results of the phase II DUX study in chemotherapy-refractory, advanced colorectal cancer. *J Clin Oncol.* 2012; 30:1505-1512.
- [25] Iida M, Brand TM, Starr MM, Li C, Huppert EJ, Luthar N, Pedersen MW, et al. Sym004, a novel EGFR antibody mixture, can overcome acquired resistance to cetuximab. *Neoplasia.* 2013; 15:1196-206.
- [26] Paz-Ares LG, Gomez-Roca C, Delord JP, Cervantes A, Markman B, Corral J, et al. Phase I pharmacokinetic and pharmacodynamic dose-escalation study of RG7160 (GA201), the first glycoengineered monoclonal antibody against the epidermal growth factor receptor, in patients with advanced solid tumors. *J Clin Oncol.* 2011; 29:3783-3790.
- [27] Freeman DJ, Ogbagabriel S, Bready J, Sun JR, Radinsky R, Hettmann T, et al. A fully human anti-HER3 mAb, demonstrates in vitro and in vivo efficacy in the FaDu model of human squamous cell carcinoma of the head and neck (SCCHN). *Mol Cancer Ther* 2011, 10(Suppl 1): abstract A182.
- [28] Falchook GS, Long GV, Kurzrock R, Kim KB, Arkenau TH, Brown MP, et al. Dabrafenib in patients with melanoma, untreated brain metastases, and other solid tumours: a phase I dose-escalation trial. *Lancet.* 2012; 379:1893-1901.
- [29] Ascierto PA, Schadendorf D, Berking C, Agarwala SS, van Herpen CM, Queirolo P, et al. MEK162 for patients with advanced melanoma harbouring NRAS or Val600 BRAF mutations: a non-randomised, open-label phase 2 study. *Lancet Oncol.* 2013; 14:249-256.
- [30] Lipson EJ, Sharfman WH, Drake CG, Wollner I, Taube JM, Anders RA, et al. Durable cancer regression off-treatment and effective re induction therapy with an anti-PD-1 antibody. *Clin Cancer Res* 2013; 19:462-8.
- [31] Gatalica Z, Snyder C, Maney T, Ghazalpour A, Holterman DA, Xiao N, et al. Programmed death 1 (PD-1) lymphocytes and ligand (PD-L1) in colorectal cancer and their relationship to microsatellite instability status. *Cancer Epidemiol Biomarkers Prev* 23:2965-70.
- [32] Manceau G1, Marisa L, Boige V, Duval A, Gaub MP, Milano G, et al. PIK3CA mutations predict recurrence in localized microsatellite stable colon cancer. *Cancer Med.* 2015; 4:371-382.
- [33] Reimers MS, Bastiaannet E, Langley RE, van Eijk R, van Vlierberghe RL, Lemmens VE, et al: Expression of HLA class I antigen, aspirin use, and survival after a diagnosis of colon cancer. *JAMA Intern Med* 174:732-739, 2014.
- [34] Tejpar S, Celik I, Schlichting M, Sartorius U, Bokemeyer C, Van Cutsem E. Association of KRAS G13D tumor mutations with outcome in patients with metastatic colorectal cancer treated with first-line chemotherapy with or without cetuximab. *J Clin Oncol.* 2012; 30:3570-3577.
- [35] Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, et al. Tumor microsatellite instability status as a predictor of benefit from fluorouracil based adjuvant chemotherapy for colon cancer. *N Engl J Med* 2003; 349:247-57.
- [36] Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J Clin Oncol* 2010; 28:3219-26.
- [37] Hutchins G, Southward K, Handley K, Magill L, Beaumont C, Stahlschmidt J, et al., Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. *J Clin Oncol.* 2011 Apr 1;29:1261-70.
- [38] Bertagnolli MM, Redston M, Compton CC, Niedzwiecki D, Mayer RJ, Goldberg RM, et al., Microsatellite instability and loss of heterozygosity at chromosomal location 18q: prospective evaluation of biomarkers for stages II and III colon cancer--a study of CALGB 9581 and 89803. *J Clin Oncol.* 2011 10;29:3153-62.
- [39] Pogue-Geile K, Yothers G, Taniyama Y, Tanaka N, Gavin P, Colangelo L, et al., Defective mismatch repair and benefit from bevacizumab for colon cancer: findings from NSABP C-08. *J Natl Cancer Inst.* 2013; 105:989-92.
- [40] Toiyama Y, Takahashi M, Hur K, Nagasaka T, Tanaka K, Inoue Y, et al., Serum miR-21 as a Diagnostic and Prognostic Biomarker in Colorectal Cancer. *J Natl Cancer Inst.* 2013 19; 105:849-59.
- [41] Song MS, Rossi JJ. The anti-miR21 antagomir, has a potential synergistic effect by perturbing an angiogenesis-associated miR30. *Front Genet.* 2014 2; 4:301.
- [42] Weisenberger DJ, Siegmund KD, Campan M, Young J, Long TI, Faasse MA, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat Genet.* 2006; 38:787-93.
- [43] Shen L, Toyota M, Kondo Y, Lin E, Zhang L, Guo Y, et al. Integrated genetic and epigenetic analysis identifies three different subclasses of colon cancer. *Proceedings of the National Academy of Sciences of the United States of America.* 2007; 104:18654-18659.
- [44] Ogino S, Noshi K, Kirkner GJ, Kawasaki T, Meyerhardt JA, Loda M, et al. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. *Gut* 2009; 58:90-6.
- [45] Barault L, Charon-Barra C, Jooste V, de la Vega MF, Martin L, Roignot P, et al. Hypermethylator phenotype in sporadic colon cancer: study on a population-based series of 582 cases. *Cancer Res.* 2008;68:8541-46
- [46] Ang PW, Loh M, Liem N, Lim PL, Grieu F, Vaithilingam A, et al. Comprehensive profiling of DNA methylation in colorectal cancer reveals subgroups with distinct clinicopathological and molecular features. *BMC Cancer.* 2010;10:227
- [47] Yagi K, Akagi K, Hayashi H, Nagae G, Tsuji S, Isagawa T, et al. Three DNA Methylation epigenotypes in human colorectal cancer. *Clin Cancer Res.* 2010; 16:21-33.