Molecular Landscape and Treatment Options for Patients with Metastatic Colorectal Cancer

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Abstract Over the last 20 years, median survival for patients with metastatic colorectal cancer (CRC) has remarkably improved from about 12 to over 30 months, mainly because of the development of new agents and patient selection using predictive biomarkers. However, the identification of the most effective treatment for an individual patient is still a challenge. Molecular profiling of CRC has made great progress, but it is limited by tumor heterogeneity and absence of driver mutation. However, RAS, BRAF, and microsatellite instability are validated biomarker recommended by NCCN and ESMO. In this review, we discuss recent advances and future developments.

Keywords Colorectal cancer · Chemotherapy · Targeted therapy · Biomarker · Personalized therapy · Molecular pathway

Introduction

Over the past several years, survival outcomes for patients with colorectal cancer (CRC) have remarkably improved because of the approval and incorporation of multiple new chemotherapeutic agents. Combined with conventional cytotoxic

and targeted drugs, overall survival (OS) has increased to over 30 months in patients with metastatic CRC (mCRC) [1–3].

Despite those improvements, identification of the most effective treatment for an individual patient is still mainly based on clinical considerations. Advances in molecular biology of CRC have led to attempts to classify CRC into subtypes based on various clinical and molecular characteristics that reflect DNA, and RNA features and protein expression [4, 5]. The Cancer Genome Atlas Network (TCGA) conducted a comprehensive molecular characterization of CRC, demonstrating the biological differences in CRC and the suggestion about therapeutic approaches to CRC. These molecularly comprehensive investigations indicate that CRC is a heterogeneous disease. Therapies targeting specific molecular characteristic show promise for CRC patients.

In this review, we focus on recent advances in the molecular characteristics of CRC, especially microsatellite instability (MSI) and somatic mutations (RAS, BRAF, and PI3KCA), next-generation sequencing (NGS) technologies, molecular classification, and their implications in treatment options for both conventional cytotoxic chemotherapy and targeted therapies for CRC.

MSI Phenotype

Microsatellite instability (MSI) is a genetic hypermutable condition that results from inactivation of the DNA mismatch repair (MMR) system. Dysfunction in genes involved in the MMR system, MLH1, MSH2, MSH6, and PMS2, results in alterations in highly repeated DNA sequences (microsatellites) [6]. A germinal mutation that inactivates MMR genes may lead to a hereditary form of MSI (“termed Lynch syndrome”). MSI is detected in approximately 15 % of sporadic CRC [7], although somatic mutations in MMR-related genes are rarely found. Methylation-induced silencing

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of MLH1 causes most sporadic CRC with MSI [8, 9]. MSI is classified into MSI-high (MSI-H), and MSI-low (MSI-L), depending on the percentage of loci that correlate to MSI characteristics. Tumors with no positive markers for MSI are designated as microsatellite stable (MSS). Sporadic MSI-H CRC tumors are associated with a specific phenotype that includes higher frequency in older people and females, proximal location, poor differentiation, mucinous and inflammatory features, and enrichment with BRAF mutations and CpG island methylator phenotype (CIMP) status [10].

Several studies have assessed the prognostic value of MSI status in CRC (Table 1), including multiple retrospective studies and meta-analyses that show MSI-H tumors to have a more favorable stage-adjusted prognosis than MSS [12, 17, 18]. A large meta-analysis of data from 10 trials with 4014 stages II–III CRC patients, including 645 with MSI, showed an association between MSI and longer OS (OR = 0.65, 95 % CI 0.53–0.79, $P < 0.0001$) relative to other CRC types [19]. This relatively favorable effect of defective MMR (dMMR) on outcomes is less pronounced in stage III CRC. For metastatic disease, a pooled analysis of four randomized first-line phase III trials (CAIRO, CAIRO2, FOCUS, and COIN) that assessed the effects of both BRAF and MSI on survival found that the progression free survival (PFS) and OS were significantly shorter for patients with dMMR tumor compared with proficient MMR (pMMR) tumors (median PFS 6.2 vs 7.6 months respectively; HR = 1.33, 95 % CI 1.12–1.57, $P = 0.001$; median OS 13.6 vs 16.8 months respectively; HR = 1.35, 95 % CI 1.13–1.61; $P = 0.001$) [20]. Given the absence of a significant interaction between BRAF mutations and dMMR, these data imply that the poor prognoses associated with MSI are driven by BRAF mutation status in these patients.

On the other hand, the predictive value of MMR status on adjuvant chemotherapy outcome is still controversial. Some studies imply that dMMR status is a predictor of decreased benefit from adjuvant 5-FU-based therapy in stage II CRC patients [12, 21]. In addition, a meta-analysis of pooled data from seven trials that included 454 MSI out of 3690 stage II–III CRC patients also showed no significant differences for recurrence-free survival (RFS: HR = 0.96, 95 % CI 0.62–1.49, $P = 0.86$) and OS (HR = 0.70, 95 % CI 0.44–1.09, $P = 0.12$) whether or not they received 5-FU based chemotherapy, whereas MSS patients had better responses to chemotherapy [22]. In contrast, a study of 1913 patients with stage II CRC from the QUASAR study showed that dMMR did not predict benefit or detrimental impact of chemotherapy [23]. Another analysis from the ACCENT database involving 7803 stage II–III CRC cases revealed that among patients with stage II CRC treated with 5-FU adjuvant chemotherapy, time to recurrence (TTR) or OS did not differ between dMMR and pMMR (TTR: HR = 0.84, 95 % CI 0.57–1.24; $P = 0.37$; OS, HR = 0.91; 95 % CI 0.63–1.31; $P = 0.62$). In stage III CRC, however, patients with dMMR cancers treated with adjuvant 5-FU

had better outcome compared to pMMR tumors (TTR: HR = 0.82, 95 % CI 0.67–0.99; $P = 0.04$; OS, HR = 0.81; 95 % CI 0.67–0.99; $P = 0.039$) [24]. In summary, because patients with stage II MSI-H tumors may have good prognoses and do not benefit from 5-FU adjuvant therapy, the NCCN panel recommends that MMR or MSI testing be performed for all patients with stage II disease, and adjuvant therapy should not be given to patients with low-risk stage II MSI-H tumors [25].

Although retrospective analyses of patients with stage III colon cancer who received adjuvant FOLFOX imply that dMMR CRCs are sensitive to oxaliplatin [26, 27], data from prospective clinical trials of oxaliplatin-based treatment are limited [28].

Significant responses to anti-PD-1 inhibitors in patients with MSI (+) cancers who failed standard chemotherapy were recently reported [29]. MSI was a significant predictor of the immune-related objective response rate (ORR; dMMR CRC 40 %; pMMR CRC 0 %) and also the immune-related PFS rate (dMMR 78 %, pMMR 11 %). The immune microenvironment of MSI CRC has been found to contain strong Th1 and CTL components not found in the vast majority of MSS tumors [30], which could significantly improve therapeutic options for mCRC [31] although the MSI rates in mCRC are very low (5 %).

Ras

RAS proteins (H-, K-, and N-Ras) are low-molecular-weight GTP-binding proteins that affect cell differentiation, proliferation, and survival [32]. Approximately 40 % of CRCs are characterized by mutations in codons 12 and 13 in exon 2 of the coding region of the KRAS gene [33–35]. A landmark finding showed that tumor KRAS mutations do not respond to anti-EGFR therapies, owing to direct activation of the MAPK signaling pathway [36]. More recent studies have also associated lower-frequency (10 %) mutations in KRAS exon 3 or 4 or in NRAS exons 2, 3, and 4 to resistance to anti-EGFR therapies [37–39]. A meta-analysis of nine randomized, controlled trials of EGFR mAbs for mCRC that evaluated RAS mutational status supported the predictive value of RAS mutational profiles for both PFS and OS [40]. Because of potential detrimental effects of anti-EGFR therapies on CRC patients whose tumors have any RAS mutations, such patients should be excluded from anti-EGFR treatments (Table 2) [40, 45].

The prognostic significance of KRAS mutations have also been evaluated; however, some systemic reviews indicate no association between KRAS mutant status and short- or long-term prognosis of patients with CRC [46]. Possibly, different mutations within the gene could have disparate prognostic influences. However, these tests are not currently recommended for prognostic purposes [25].

Table 1 Clinical trials that evaluated the prognostic and predictive significance of mismatch repair status in colorectal cancer

Study [reference]	Stage	Treatment	MMR status	N	5-year DFS/TTR /RFS (%)	HR	95 % CI	P	5-year OS (%)	HR	95 % CI	P
Sinicropo et al. [11]	II/III		dMMR	344	72	0.73	0.59–0.91	0.004	77	0.73	0.59–0.90	0.004
			pMMR	1797	63				70			
Sargent et al. [12]	II/III	Surgery alone	dMMR	37	76	0.46	0.22–0.95	0.03	81	0.51	0.24–1.10	0.06
			pMMR	191	53				62			
		5-FU	dMMR	33	71	0.90	0.44–1.82	0.77	75	0.73	0.35–1.54	0.41
			pMMR	196	64				71			
ACCENT [13]	II	Surgery alone	dMMR	63	89	0.35	0.15–0.80	0.013	90	0.37	0.17–0.81	0.013
			pMMR	244	74				78			
		5-FU	dMMR	235	88	0.84	0.57–1.24	0.37	88	0.91	0.63–1.31	0.62
			pMMR	920	83				87			
	III	Surgery alone	dMMR	37	60	0.79	0.45–1.39	0.41	59	0.84	0.49–1.43	0.51
			pMMR	277	47				54			
		5-FU	dMMR	390	72	0.82	0.67–0.99	0.04	77	0.81	0.67–0.99	0.039
			pMMR	2333	64				71			
NSABP C-07 [14]	II/III	5-FU FLOX	dMMR	86	NA	1.01	0.45–2.25	0.98	NA	NA	NA	NA
			pMMR	85	NA				NA	NA	NA	NA
		5-FU FLOX	dMMR	635	NA	0.82	0.67–1.00	0.054	NA	NA	NA	NA
			pMMR	675	NA				NA	NA	NA	NA
MOSAIC [15]	II/III	5-FU FOLFOX	dMMR	51	74	0.48	0.21–1.12	0.088	82	0.41	0.16–1.07	0.069
			pMMR	44	86				91			
		5-FU FOLFOX	dMMR	442	65	0.87	0.70–1.07	0.185	79	0.91	0.72–1.15	0.428
			pMMR	471	72				81			
N0147 [16]			dMMR	314	NA	0.82	0.64–1.04	0.1055	NA	0.83	0.63–1.09	0.1822
			pMMR	2266	NA				NA			

Table 2 Summary of phase III trial according to RAS mutational status

Study	Phase	RAS type	Therapy	n	PFS (M)	HR	95 % CI	P	OS (M)	HR	95 % CI	P	P ORR (%)	P
OPUS [41]	II	KRAS ^{Wt}	FOLFOX + Cmab	82	8.3	0.57	0.38–0.86	0.0064	22.8	0.86	0.60–1.22	0.39	57	0.0027
			FOLFOX	97	7.2									
CRYSTAL [42]	III	All RAS ^{Wt}	FOLFOX + Cmab	38	12.0	0.53	0.27–1.04	0.0615	19.8	0.94	0.56–1.56	0.80	58	0.0084
			FOLFOX	49	5.8				17.8				29	
		KRAS ^{Wt}	FOLFIRI + Cmab	316	9.9	0.70	0.56–0.87	0.0012	23.5	0.80	0.67–0.95	0.0093	57	<0.001
			FOLFIRI	350	8.4	0.56	0.41–0.76	<0.001	20.0	0.69	0.54–0.88	0.0024	40	<0.001
PRIME [38]	III	All RAS ^{Wt}	FOLFIRI + Cmab	178	11.4	0.56	0.41–0.76	<0.001	28.4	0.83	0.70–0.98	0.03	NA	NA
			FOLFIRI	189	8.4				20.0				66	<0.001
		KRAS ^{Wt}	FOLFOX + Pmab	325	9.6	0.80	0.66–0.97	0.02	23.8	0.77	0.64–0.94	0.009	NA	NA
			FOLFOX	331	8.0				19.4				NA	NA
PEAK [43]	III	All RAS ^{Wt}	FOLFOX + Pmab	259	10.1	0.72	0.58–0.90	0.004	25.8	0.62	0.44–0.89	0.009	58	NA
			FOLFOX + Bmab	253	7.9	0.87	0.65–1.17	0.353	34.2	0.63	0.39–1.02	0.058	64	NA
		All RAS ^{Wt}	FOLFOX + Bmab	142	10.9	0.65	0.44–0.96	0.029	24.3	0.77	0.62–0.96	0.017	62	0.18
			FOLFOX + Pmab	143	10.1				41.3				61	
FIRE-3 [3]	III	KRAS ^{Wt}	FOLFOX	88	13.0	1.06	0.88–1.26	0.55	28.9	0.70	0.53–0.92	0.011	65	0.32
			FOLFIRI + Cmab	82	9.5	0.93	0.74–1.17	0.54	28.7	0.92	0.78–1.09	0.34	60	0.02
		All RAS ^{Wt}	FOLFIRI + Bmab	297	10.0	1.04	0.91–1.17	0.55	25.0	0.9	0.7–1.1	0.40	69	<0.01
			FOLFIRI + Cmab	295	10.3				33.1				54	
CALGB/SWOG 80405 [44]	III	KRAS ^{Wt}	FOLFIRI + Bmab	171	10.4	1.10	NA	0.31	25.6	0.9	0.7–1.1	0.40	69	<0.01
			FOLFIRI + Cmab	171	10.2				29.9				57	
		All RAS ^{Wt}	FOLFIRI/FOLFOX + Cmab	578	10.4				32.0				54	
			FOLFIRI/FOLFOX + Bmab	599	10.8				31.2					

PFS progression free survival, M months, HR hazard ratio, CI confidence interval, OS overall survival, ORR objective response rate, Wt wild-type, Cmab cetuximab, Pmab panitumumab, Bmab bevacizumab

Retrospective data from large phase III trials suggested that patients who carry the KRAS G13D mutation might benefit from cetuximab [47] and panitumumab [48] in first and advanced treatment lines [49], which implied that the KRAS G13D mutation is clinically significant. Because of these results, two prospective phase II trials [50, 51] were performed; however, neither trial showed activity from cetuximab monotherapy in patients with KRAS G13D-mutated mCRC. Currently, anti-EGFR therapy for patients whose tumors have G13D mutations is not routinely recommended.

Even among patients with RAS wild-type (wt) mCRC that initially responds to anti-EGFR therapies, essentially all eventually develop secondary resistance to these agents through numerous mechanisms. Diaz et al. assessed circulating tumor DNA (ctDNA) in the blood of mCRC patients during panitumumab therapy and found that preexisting low-abundance KRAS-mutant clones may be selected through anti-EGFR treatment [52]. Additionally, Bertotti et al. used large-scale genomic and targeted therapeutic analyses of CRC patients who underwent anti-EGFR therapy to show that insulin receptor substrate 2 (IRS2) mediates a novel mechanism of sensitivity to anti-EGFR therapy [53]. IRS2 is regulated by insulin receptor (IR)/insulin-like growth factor (IGF) receptors [54]. IRS2 amplification and mutations were identified in tumors with increased sensitivity to anti-EGFR therapy. Another apparent mechanism of resistance to cetuximab is an EGFR extracellular domain (ECD) mutation that prevents binding to cetuximab but not to panitumumab [55]. The EGFR S492R mutation alters the epitope by which cetuximab binds EGFR, but because panitumumab binds a different epitope than cetuximab, patients with these mutations might benefit from subsequent panitumumab therapy.

Some new *EGFR* inhibitors appear to overcome resistance to cetuximab or panitumumab due to the emergence of EGFR ECD mutations. MM-151 is a third-generation EGFR inhibitor that uses three distinct antibodies that bind different EGFR epitopes to block potentially signaling downstream of EGFR, despite escalating doses of EGF [56]. A preclinical study showed MM-151 to inhibit cell signaling and proliferation in cells from a patient whose tumor developed an EGFR ECD mutation during cetuximab treatment [57]. Moreover, after MM-151 treatment, liquid biopsies of patients with EGFR ECD mutations at baseline showed decreased or stabilized EGFR ECD mutant DNA concentrations that paralleled response assessments after using radiological methods.

Sym004 is another mixture of two synergistic nonoverlapping anti-EGFR antibodies that target different epitopes of the EGFR ECD [58]. Sym004 synergistically induces EGFR internalization and degradation in vitro and inhibits tumor growth in vivo [59]. Colorectal tumors of patients who develop secondary resistance to EGFR blockade often display heterogeneous resistance mechanisms, which would presumably be insensitive to EGFR-targeted monotherapy, including these

new agents. Additional work is needed to elucidate relationships among these concurrent mechanisms of resistance.

BRAF

The BRAF protein is a serine/threonine kinase, downstream of KRAS, which acts as signal transducer from the membrane to the nucleus [60], and is mutated in approximately 10 % of CRC [61]. The most common BRAF mutation is at position V600E and causes a constitutive activation of the MAPK pathway [62]. In addition, BRAF mutations are mutually exclusive with RAS mutations [63]. In CRC, BRAF mutations are found more commonly in tumors with proximal locations, poor differentiation, mucinous histology, CIMP, or MSI [64, 65]. Results from a recent systematic review indicate that active BRAF mutations occur mostly in tumors with proximal location (OR 5.22; 95 % CI 3.80–7.17; $P < 0.001$), T4 grade (OR 1.76; 95 % CI 1.16–2.66; $P = 0.007$), and/or poor differentiation (OR 3.82; 95 % CI 2.71–5.36; $P < 0.001$) [66].

Evidence from retrospective analyses and randomized clinical trials has associated BRAF mutations with poor prognosis in patients with pMMR tumors (Table 3) [75, 76]. A prospective analysis of tissues from patients with stage II–III colon cancer enrolled in the PETACC-3 trial showed that the BRAF mutation is prognostic for shorter OS in patients with MSI-L or MSS tumors (HR 2.2; 95 % CI 1.4–3.4; $P = 0.0003$) [75]. A recent meta-analysis also showed that patients with advanced RAS-wt/BRAF-mutant CRC did not benefit from cetuximab or panitumumab treatments [77]. BRAF mutation status cannot be shown to predict benefit from cetuximab treatment [67]. Therefore, this testing is currently optional and not a necessary part of deciding whether to use anti-EGFR agents.

BRAF inhibitors, such as vemurafenib and dabrafenib, have produced dramatic response rates of 50–80 % in BRAF V600 mutant melanoma. In contrast, only a 5 % response rate was seen in patients treated with vemurafenib who had metastatic CRC that harbored the same BRAF V600 mutation [78, 79]. Corcoran et al. investigated this differential outcome and found that MAPK suppression by BRAF inhibitor alone in BRAF-mutant CRC cells was transient, followed by rapid reactivation of MAPK signaling and re-accumulation of phosphorylated ERK, despite continued presence of the drug [80]. Therefore, combination strategies to inhibit both BRAF and EGFR, or other approaches that target interacting pathways (i.e., that co-targets BRAF plus MEK or PI3K), are required. Other findings highlighted hyperactivation of the PI3K/Akt/PTEN pathway as a mechanism of resistance to BRAF inhibition in CRC [79, 81]. Vemurafenib with an AKT inhibitor was found to inhibit growth of BRAF-mutant CRC xenografts in a murine model [79]. Ongoing trials in patients with BRAF-mutant CRC will provide important insights on this issue (Table 4).

Table 3 Summary of clinical trials in BRAF mutant tumor

Treatment line	Trial (reference)	BRAF mutation	Regimen	N	PFS (M)	HR	95 % CI	P	OS (M)	HR	95 % CI	P	ORR (%)	P
1st	CRYSTAL [67]	6 %	FOLFIRI + Cnab FOLFIRI	26 33	8.0 5.6	0.93	0.43–2.06	0.87	14.1 10.3	0.91	0.51–1.62	0.74	15.2 19.2	0.91
1st	OPUS [68]	4 %	FOLFOX + Cnab FOLFOX	6 5	NA	NA	NA	NA	20.7 4.4	NA	NA	NA	NA	NA
1st	PRIME [38]	8 %	FOLFOX + Pnab FOLFOX	24 29	6.1 5.4	0.58	0.29–1.15	0.12	10.5 9.2	0.90	0.46–1.76	0.76	NA	NA
1st	COIN [69]	8 %	FOLFOX/XELOX + Cnab FOLFOX/XELOX	45 57	NA	NA	NA	NA	7.2 10.0	1.18	0.76–1.81	0.46	NA	NA
1st	FIRE-3 [70]	12 %	FOLFIRI + Cnab FOLFIRI + Bnab	23 25	4.9 6.0	0.87	0.49–1.57	0.65	12.3 13.7	0.87	0.47–1.61	0.65	52.2 40.0	0.29
2nd	20050181 [71]	8 %	FOLFIRI + Pnab FOLFIRI	22 23	2.5 1.8	0.69	0.32–1.49	0.34	4.7 5.7	0.64	0.32–1.28	0.20	NA	NA
2nd>	PICCOLO [72]	15 %	CPT-11 + Pnab CPT-11	31 37	NA	1.40	0.82–2.39	NA	NA	1.84	1.10–3.08	NA	6.5 10.8	NA
2nd>	CO.17 [73]	5 %	Cnab BSC	4 6	NA	0.76	NA	0.69	1.8 3.0	0.84	0.20–3.58	0.81	0	NA
3rd	20020408 [74]	12 %	Pnab BSC	9 6	NA	0.34	0.09–1.24	NA	NA	NA	NA	NA	0	NA

PFS progression-free survival, M months, HR hazard ratio, CI confidence interval, OS overall survival, ORR objective response rate, wt wild-type, Cnab cetuximab, Pnab panitumumab, Bnab bevacizumab, NA not available

Table 4 Recent and ongoing clinical trials of BRAF inhibitor for mCRC

Strategy	Therapy	Genomic profile	Phase	N	ORR (%)	DCR (%)	PFS (M)	OS (M)	Clinical development	Reference
BRAF monotherapy	Vemurafenib	BRAF ^{Mut}	I	21	5	84	2.1	7.7	Complete	NCT00405587 [78]
BRAF monotherapy	Vemurafenib BRAF ^{Mut}	BRAF ^{Mut}	II	10	0	50	4.5	9.3	Complete	NCT01524978 [82]
BRAF + MEK	Dabrafenib + trametinib	BRAF ^{Mut}	I/II	43	12	63	3.5	NA	Complete	NCT01726738 [83]
BRAF + EGFR	Vemurafenib + cetuximab	BRAF ^{Mut}	II	27	4	73	3.7	7.1	Complete	NCT01524978 [82]
BRAF + EGFR	Vemurafenib + panitumumab	BRAF ^{Mut}	Pilot	15	13	67	3.2	7.6	Complete	NCT01791309 [84]
BRAF + EGFR	Encorafenib + cetuximab	BRAF ^{Mut} + KRAS ^{wt}	I/II	26	23	NA	3.7	NA	Ongoing	NCT01719380 [85]
BRAF + EGFR	Dabrafenib + panitumumab	BRAF ^{Mut}	I/II	20	10	90	NA	NA	Ongoing	NCT01750918 [86]
BRAF + EGFR + MEK	Dabrafenib + panitumumab + trametinib	BRAF ^{Mut}	I/II	24	21	83	NA	NA	Ongoing	NCT01750918 [86]
BRAF + EGFR + PI3K	Encorafenib + cetuximab + alpelisib	BRAF ^{Mut} + KRAS ^{wt}	I/II	28	25	NA	4.3	NA	Ongoing	NCT01719380 [85]
BRAF + EGFR + CT	Vemurafenib + cetuximab + irinotecan	BRAF ^{Mut}	I	12	44	89	NA	NA	Ongoing	NCT01787500 [87]
BRAF + EGFR + CT	Vemurafenib + cetuximab + irinotecan	BRAF ^{Mut}	II (RCT)	78*	NA	NA	NA	NA	Ongoing	NCT02164916
BRAF + EGFR + WNT	Encorafenib + cetuximab + WNT974	BRAF ^{Mut} + WNT ^{wt}	I/II	60*	NA	NA	NA	NA	Ongoing	NCT02278133

Recent data suggests that FOLFOXIRI + bevacizumab (Bmab) might be a reasonable first-line treatment for BRAF-mutant mCRC [88, 89]. Although its treatment effect did not significantly differ from that of FOLFIRI + Bmab, its survival HRs were OS of 0.54 (95 % CI: 0.24–1.20) and PFS of 0.57 (95 % CI 0.27–1.23) compared with FOLFIRI + Bmab.

PIK3CA

As with the MAPK pathway, EGFR signaling is mediated also by the PI3K-AKT pathway which is important for cancer cell survival [90]. PI3KCA is a heterodimeric protein, composed of a regulatory subunit (p85) and a catalytic subunit (p110); it functions as a lipid kinase. PIK3CA mutations are present in approximately 15 % of CRCs [34]; most of these mutations are single amino-acid substitutions located in hot spots in the helical (exon 9) or kinase domains (exon 20) [91]. These mutations may also affect anti-EGFR therapy responsiveness. Mao et al. conducted a systematic review and meta-analysis to investigate the association between PIK3CA mutations and resistance to anti-EGFR therapy in mCRC, by mutated PIK3CA exon. Their systematic review of 13 studies showed that PIK3CA mutation alone did not correlate with survival of patients with metastatic disease. Among patients with KRAS-wt mCRC who underwent anti-EGFR therapy, PIK3CA mutation on exon 20 was related to worse prognosis, but not significantly so. This result suggests that PIK3CA exon 20 mutations is a biomarker for resistance to anti-EGFR therapy in KRAS-wt mCRC; further studies are needed to confirm this relation and clarify its clinical implications.

In addition to the adjuvant setting, two large observational studies suggested that aspirin use was associated with significantly increased survival in patients with PIK3CA-mutant CRC but not PIK3CA-wt CRC [92, 93]. The underlying mechanism behind the benefit of aspirin in this setting is unclear. PI3K mutations result in constitutive activation of the AKT pathway, which enhances NF- κ B, a transcription factor that plays a pivotal role in upregulating pro-inflammatory genes, including COX-2. PI3KCA mutations may also activate signaling of Wnt, which is an essential oncogenic pathway in CRC. Aspirin inhibits activity of both NF- κ B and the Wnt/ β -catenin pathway; patients with PIK3CA-mutant CRC may therefore benefit from this mechanism.

Molecular Classification of CRC

The development of convincing NGS technologies has revolutionized DNA sequencing by reducing both its time and cost in the past few years, offering the opportunity for a comprehensive description of the different CRC subtypes [94]. Pivotal comprehensive study conducted by TCGA showed the first tumor dataset with complete molecular measurements, including exome sequencing, DNA copy number,

promoter methylation, and mRNA and miRNA expression analysis [4, 95]. According to whole genome sequencing, 16 % were found to be hypermutated, which were associated with MSI and/or CIMP. Among the nonhypermutated cancers, the four most frequently mutated genes are APC (81 %), p53 (59 %), KRAS (50 %), and PIK3CA (18 %). Among the hypermutated cancers, the four most frequently mutated genes are ACVR2A (63 %), APC (51 %), TGFBR2 (51 %), and BRAF (46 %). The WNT signaling pathway was altered in 93 % of all tumors, including biallelic inactivation of APC or activating mutations of CTNNB1. Furthermore, genomic alterations involved in TGF-beta signaling pathway were found in 27 % of the nonhypermutated and 87 % of the hypermutated tumors. Recurrent genetic alterations are eventually grouped according to the activity of four pathways (WNT, MAPK-PI3K, TGF-B, and p53) by hypermutation status. The findings from the TCGA have provided a useful resource for understanding CRC and, at the same time, have required the development of new classification schemes.

Recently, an international consortium has proposed a gene-expression-based subtyping classification system for CRC that defines four consensus molecular subtypes (CMSs) of CRC with distinguishing features: CMS1 (MSI-immune, 14 %), demonstrating hypermutation, MSI, and strong immune activation; CMS2 (canonical, 37 %), epithelial tumors with marked activation of WNT and MYC signaling; CMS3 (metabolic, 13 %), epithelial tumors with evident metabolic dysregulation; and CMS4 (mesenchymal, 23 %), with prominent activation of TGF β signaling, stromal invasion, and angiogenesis [5]. In addition, the CMS groups have important associations with some clinical variables and prognosis. The next step should be to link these subtype classifications to response to therapy to identify predictive biomarkers.

Liquid Biopsy

This new genetic technology has allowed to identify cell-free circulating tumor DNA (ctDNA) and RNA (ctRNA) which are released from tumor cells into bloodstream. A BEAMing digital PCR technology (beads, emulsion, amplification, and magnetics) is the methods of detecting somatic mutations in small amounts of ctDNA. This system amplifies nucleic acids in the presence of magnetic beads and assesses its quantity by using a flow cytometry [96], allowing to identify genetic variations of ctDNA, but also to precisely quantify their number in comparison to the number of wild-type sequences [97]. However, this method interrogates only a few loci, and mutations in genes that lack mutational hotspots, such as tumor suppressors, are missed. On the other hand, NGS technologies have been used in the field of liquid biopsy. The main advantage of NGS of a liquid biopsy is that this approach is applicable to all patients because it does not rely on recurrent genetic changes [98].

Many studies suggested that ctDNA levels might be useful for monitoring patients with CRC and for recognizing individuals with high-risk recurrence [99]. Frattini et al. showed that ctDNA levels were significantly higher in patients with CRC; they decreased during the follow-up period in tumor-free patients and increased again at tumor recurrence [100]. These results are in line with the findings of Spindler et al. which demonstrated that median ctDNA levels were significantly higher in mCRC compared to healthy individuals and that confirmed a shorter OS with increasing levels of baseline ctDNA [101]. The correlation between the ctDNA level and clinical response was also assessed in a retrospective study of the CORRECT study cohort [102]. Although a high ctDNA level does not predict regorafenib clinical response, ctDNA level was found as prognostic factor, with shorter median survival observed in patients with a higher ctDNA level than in patients with a lower ctDNA level regardless of treatment.

The detection of somatic point mutations in ctDNA was reported by in 1994 [103]. Technological advances have improved the analytical sensitivity of detection RAS and BRAF mutations in the plasma samples of mCRC patients [104–106]. KRAS mutant fragments were detected in the blood of patients with KRAS-mutant colorectal tumors, with high specificity (98 %) and sensitivity (92 %) [107]. These results suggest that the “liquid biopsy” is a feasible alternative to a solid tissue biopsy for identifying specific mutations. When tumor tissue specimens from metastatic cancer patients are unavailable, liquid biopsies offer an alternative that can be rapidly implemented without the pain, risk, and expense entailed by a biopsy of one of the metastatic lesions [105].

Furthermore, liquid biopsies allow the identification of resistance mutations that occur when patients first respond to therapy and then progress [108, 109]. Diaz et al. showed that 38 % of patients whose tumor were initially KRAS wild type developed detectable mutation in KRAS in their serum. In the same way, Bettegowda et al. reported that 96 % of patients, who objectively responded to anti-EGFR therapy but subsequently relapsed, developed one or more mutations in genes involved in the mitogen-activated protein kinase pathway [105]. These results suggest that liquid biopsy could detect the emergence of KRAS mutant clones months before radiographic progression [110].

Conclusions

With increasing number of new drugs in clinical studies and development of new technologies such as liquid biopsies, personalized therapies are becoming more and more a reality. We begin to understand that CRC is a heterogeneous disease with no driver mutations and most of the genetic aberrations are not sufficient to guide treatment decisions. We only have a few predictive and

prognostic markers we can clinically use. In the future, NGS technology will help our progress toward understanding CRC genomes and molecular classifications, which will lead to a better personalized targeted therapeutics for CRC management. There is growing need for translational research focused on the early identification of biomarkers, and predictive biomarkers require even more extensive data for validation derived from large randomized clinical trials and meta-analysis.

Compliance with Ethical Standards

Conflict of Interest HJ Lenz has received honoraria from Merck Serono, Roche, Celgene, Bayer, and Boehringer Ingelheim. The other authors have no conflict of interest.

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