Colorectal Cancer Liver Metastases

Subjects: Oncology

Contributor: Yuliya Kitsel , Timothy Cooke , Vlasios Sotirchos , Constantinos Sofocleous

Colorectal cancer (CRC) is a leading cause of death among cancer patients, and the liver is the most common visceral metastatic. Molecular cancer biomarkers are any measurable molecular indicator of the risk of cancer, the occurrence of cancer, or patient outcome, to help personalize treatment and to identify patients who may benefit most from a specific therapy. They may include germline or somatic genetic variants, epigenetic signatures, transcriptional changes, and proteomic signatures.

colorectal cancer liver metastases biomarkers

1. Introduction

Colorectal cancer (CRC) is the third-most common form of cancer with the fourth highest mortality rate worldwide [1]. Over half of CRC patients undergoing autopsy have been found to have liver metastases, suggesting that colorectal cancer liver metastases (CRLM) are a major cause of death in this population [2]. Despite promising advances in chemotherapy and liver-directed locoregional therapies (i.e., surgery, ablation, and arterially directed treatments), recurrences with eventual progression of disease and liver failure are common [3].

2. Genetic Alterations in Colorectal Cancer

Cancers are caused by the accumulation of mutations in genes that change the normal programming of cellular differentiation, proliferation, and death. Through the development of DNA sequencing, the understanding of the DNA changes associated with CRC is rapidly growing [4]. The ability to identify sequence variants in genes has improved the understanding of how cancer develops, allowing the development and application of treatments targeting these variants [4].

The Cancer Genome Atlas (TCGA) was created to study genomic changes in various cancer forms [4][5]. Each individual CRC contains between 60 and 1500 mutations. Few of these mutations have been found to be clinically relevant [6]. CRC generally divides biologically to those types exhibiting microsatellite instability (MSI) and those that are stable.

According to the TCGA, in non-hypermutated tumors (mutation rates of ≤12 per 10^6 base pairs), the most commonly mutated genes were APC, TP53, KRAS, PIK3CA, NRAS, SMAD4, FBXW7, and TCF7L2 [7]. Mutated NRAS and KRAS genes typically displayed oncogenic mutations of codon 61 or codon 12 and 13 [7]. In hypermutated tumors, TGFBR2, APC, ACVR2A, MSH3, MSH6, TCF7L2, SLC9A9, and BRAF (V600E) were

frequently mutated [7]. However, the APC and TP53 genes were more commonly mutated in non-hypermutated tumors versus hypermutated tumors: APC (81 vs. 51%) and TP53 (60 vs. 20%) [7]. These results suggest that hypermutant and non-hypermutant tumors advance through different series of genetic events.

Changes in MAPK, WNT, PI3K, TGF-b, and p53 signaling pathways are present in CRC [7]. WNT is the most prominent with regards to the carcinogenesis of colorectal cancer [8]. WNT signaling regulates the amount of β-catenin through processes that involve ubiquitin-mediated degradation and phosphorylation, thus impacting overall signal transduction [9]. Loss of adenomatous polyposis coli (APC), a negative regulator of the WNT pathway, is the hallmark of human CRC [9].

Different clinical and biologic characteristics have been observed between left-sided (arising from the splenic flexure, descending, or sigmoid colon) versus right-sided (arising from the cecum, ascending colon, hepatic flexure, or transverse colon) primary tumors. Genetic expression patterns differ between the right- and left-sided colonic epithelium, including a higher expression in cytochrome P-450 in the right colon, suggesting varying levels of exposure to ingested metabolites [10][11]. Overall, right-sided colon cancers carry a worse prognosis when compared to left-sided tumors and are more likely to have advanced TNM staging, peritoneal dissemination, MSI leading to a hypermutated state, BRAF/KRAS/PIK3Ca/SMAD4/FBXW7 mutations, and hypermethylation via the CpG island methylator phenotype (CIMP) [12][13][14]. On the other hand, mutations in TP53 and APC are often seen in left-sided CRC [15][16]. In addition to point mutations, left-sided CRC express more receptor tyrosine kinase amplifications, such as those in epidermal growth factor receptor (EGFR) and ERBB2 [10]. The variations in CRC sidedness can also have embryonic roots: the right side of the colon develops from the midgut, whereas the left side develops from the hindgut, influencing blood flow [17]. Lastly, gut microbiome differences can participate in the phenotypic expression of CRC sidedness [15].

Classifying tumor aggressiveness is based on several variables including the ability to create distant metastasis, lymph node status, tumor stage, and vascular invasion. Tumor aggressiveness has been associated with certain localized amplifications, deletions, and gene expression modification, including those of SCN5A, a well-known regulator of colon cancer [7]. Certain gene mutations also carry prognostic significance [18]. For example, highest cure rates have been observed in patients with NOTCH1 and PIK3C2B mutations, compared to patients with SMAD3 mutations, who had the lowest rates of cure [19].

3. Molecular Biomarkers of Colorectal Cancer

In the 1980s, the median overall survival (OS) of patients diagnosed with metastatic CRC (mCRC) did not exceed six months. The development and implementation of biomarkers, combined with significant advances in chemotherapeutics and locoregional treatments, have increased OS to a median of almost 30 months in the same population [20][21][22][23][24][25]. Elevated carcinoembryonic antigen (CEA) levels have been associated with poor prognosis and shorter OS following CRC resection. Of note, CEA levels can be elevated in other malignancies and inflammatory states, and therefore CEA is not a specific diagnostic biomarker [26]. Lack of normalization of CEA levels following resection is likely the result of inadequate resection and presence of metastatic disease. Routine

CEA monitoring can help identify early, recurrent, or metastatic disease, that may benefit from additional therapies [27]. CEA is considered a sensitive indicator of post treatment recurrence [27].

Currently, the National Comprehensive Cancer Network (NCCN) recommends testing for the following gene mutations in patients with CRC, as they are potentially actionable biomarkers that can guide therapeutic considerations: BRAF and KRAS/NRAS mutations, HER2 amplifications, and microsatellite instability high (MSI-H)/mismatch repair (MMR) (**Table 1**) [28].

Table 1. NCCN recommended biomarkers for CRC.

Biomarkers	Clinical Implication
KRAS/NRAS	Anti-EGFR therapy are not indicated for the treatment of patients harboring NRAS or KRAS mutations.
BRAF V600E	Poor prognosis regardless of treatment. Prognostic marker for BRAF-targeted therapy.
	Related to sporadic CRC, excludes Lynch syndrome.
MSI/dMMR	Improve patient prognosis. dMMR's ties to Lynch syndrome. Prognostic marker for checkpoint inhibitors therapy. dMMR status is a strong negative predictor of 5-fluorouracil efficacy.
HER2	Predictive biomarker for HER2-targeted therapy in patients with wild-type RAS and BRAF tumors. Predict resistance to monoclonal antibodies that target EGFR.

3.1. KRAS and NRAS Mutations

RAS proteins are GTPases that regulate cell survival and proliferation. Human RAS genes, including Kirsten RAS (KRAS), Harvey RAS (HRAS), and neuroblastoma RAS (NRAS), are integral in GTPase activity [29]. Oncogenic mutations in RAS signaling pathways are associated with different aspects of cancer development. Since oncogenic mutations in various components of the RAS/MAPK pathway seem to be mutually exclusive in many tumors, deregulating RAS-dependent signaling is essential in tumorigenesis [30]. Regarding the prevalence of RAS mutations in CRC patients, a recent study showed that just over 50% of patients had KRAS mutations, with HRAS and NRAS mutations occurring at lower rates [31]. RAS signaling is not unique for CRC, as it is also present in non-malignant conditions such as diabetes, autoimmune, and inflammatory disorders [30].

Because the RAS/RAF/MEK/ERK pathway is downstream of EGFR, there are significant considerations that must be made regarding treatment of tumors expressing KRAS or NRAS mutations. Mutations within this pathway are robust negative predictive markers, and treatment with panitumumab or cetuximab is ineffective for tumors that have mutations in exons 2, 3, or 4 of the NRAS or KRAS genes [32][33][34][35][36][37]. Furthermore, these same mutations have been shown to express resistance to anti-EGFR therapy, both with and without chemotherapy, and

correlate with worse outcomes [31][38][39][40][41]. Nevertheless, patients with left-sided CRC wild type RAS tumors have excellent outcomes when treated with anti-EGFR based therapy [42].

The NCCN Colon and Rectal Cancers Panel recommends determination of the RAS mutation status at diagnosis of stage IV disease [28]. Consistent with these recommendations, the American Society for Clinical Pathology (ASCP), Association for Molecular Pathology (AMP), College of American Pathologists (CAP), and the American Society of Clinical Oncology (ASCO) also developed a guideline on molecular biomarkers for CRC that also recommends RAS (KRAS/NRAS) genotyping of tumor tissue in all patients with mCRC [43]. KRAS and NRAS codons 12 and 13 (exon 2), 59 and 61 (exon 3), and 117 and 146 (exon 4) should all be included in mutational analysis [44][45]. Either the primary tumor or liver metastases can be tested for KRAS mutation analysis since there is a 96.4% concordance of mutational status between the primary sites and a metastasis [43]. Cetuximab or panitumumab are not indicated for the treatment of patients harboring NRAS or KRAS mutations, regardless of whether the drugs are used in isolation or with other anti-cancer therapies [46] since they will not respond to such agents. This minimizes toxicity and improves treatment cost-effectiveness. RAS genotyping of CRC at stage I, II, or I II is not recommended, and RAS testing should not be used for regimen selection in the first-line setting [43][47][48].

3.2. BRAF V600E Mutations

BRAF is a protein kinase downstream of RAS protein in the RAS-RAF-MEK-ERK kinase in the EGFR pathway [13][49]. RAF genes code for cytoplasmic serine/threonine protein kinase activity after binding RAS protein [50]. The RAS/RAF/MEK/MAP kinase pathway is an essential mechanism of tumor cell proliferation that mediates cellular responses to growth signals [51][52]. BRAF mutations, specifically with the V600E variant, occur in several malignancies [53]. In mCRC, BRAF mutations are found in 8–12% of patients (in 90% with V600E). BRAF-mutated tumors are more frequently seen in elderly and female patients [13][51][54][55][56]. Since BRAF is downstream of RAS, BRAF V600E mutations makes response to cetuximab or panitumumab highly unlikely, unless administered with a BRAF inhibitor [49][57][58].

At diagnosis of stage IV disease, NCCN recommends BRAF genotyping of either the primary or a metastatic tumor site as a predictive and prognostic marker for BRAF-targeted therapy [28][54][55]. BRAF V600E mutations have a poor prognosis regardless of treatment [54]. BRAF V600E mutation testing can be performed via direct DNA sequence analysis on formalin-fixed paraffin-embedded tissues and PCR amplification. Other methods for detecting this mutation include allele-specific polymerase chain reaction, immunohistochemistry (IHC), or next generation sequencing (NGS) [28].

A lower median OS for patients with BRAF-mutant mCRC compared to patients with wild-type BRAF (10.4 months vs. 34.7 months) has been reported [13]. Moreover, recurrences of resected stage III colon cancer had significantly worse survival in BRAF mutated tumors [59].

MSI-H/dMMR status is also strongly associated with the BRAF V600E mutation, with approximately 60% of MSI-H tumors having a BRAF mutation and only 5–10% of Microsatellite Stable (MSS) tumors having the same mutation

in sporadic CRCs [60][61]. Because the BRAF V600E mutation is related to sporadic CRC patients but excludes Lynch syndrome, it is a key biomarker in distinguishing between the two etiologies [62].

In creating guidelines for CRC molecular biomarkers, the ASCP, CAP, AMP, and ASCO recommended that patients with dMMR tumors with loss of MLH1 receive BRAF p.V600 mutational analysis to evaluate for risk of Lynch syndrome. Although the absence of BRAF mutation does not completely exclude the risk of Lynch syndrome, the presence of a BRAF mutation would strongly favor a sporadic etiology [44].

3.3. Microsatellite Instability (MSI)/DNA Mismatch Repair Deficiency (dMMR) Status

Microsatellites are short segments of DNA that repeat at specific genomic locations. Under normal circumstances, in the event of an insertion or deletion within these regions, the MMR system rectifies any error. However, defects in this system cause deficient mismatch repair (dMMR). Although tumors with MSI retain their chromosomal number, they contain microsatellites that vary in length due to dMMR, which is thought to be a principle of early tumorigenesis [63]. About 15% of CRCs are affected by dMMR, which is more frequently seen in early stages of the disease (15–20% in stages I–II, 10–15% in stage III, and just 5% in stage IV) [64][65]. Up to 20% of patients with sporadic CRC have dMMR which is usually caused by hypermethylation of the MLH1 promoter [66][67][68].

Despite the numerous mutations present in dMMR tumors, mutant proteins are able to circumvent the immune system by binding to the T-effector cell's programmed cell death protein (PD-1) receptor via programmed-death ligands 1 and 2 (PD-L1 and PD-L2) [69]. This system was initially thought to be protective for the host, but tumor cells that upregulate PD-1 have been shown to be elusive to the immune system [69]. As a result, it was theorized that dMMR tumors may have sensitivity to PD-1 inhibitors. This was validated in a small prospective phase 2 study, that showed complete response in 12 dMMR patients with stage II–III rectal cancer treated with single-agent dostarlimab (an anti-PD-1 monoclonal antibody), without any resection or radiotherapy [70]. There was no evidence of tumor in any patients after a minimum 6-month follow-up period [70]. In MSI-H CRCs, a recent study established a link between inflammatory states and suboptimal tumor response to PD-1 inhibition; an elevated neutrophil-to-lymphocyte ratio indicated poor tumor response to checkpoint inhibitors [71].

As a result, the NCCN now recommends MSI or MMR testing for any patient with a history of CRC, and checkpoint inhibitors for dMMR/MSI-H disease [28]. dMMR/MSI-H testing is essential for four main reasons. Firstly, because of the potential connection between dMMR and Lynch syndrome, it is a potent screening test to identify the most common cause of hereditary CRC [72]. Secondly, identifying dMMR status has proven to be one of the best ways to improve patient prognosis, particularly in those with stage II CRC, where MSI-H was associated with longer OS, relapse-free survival, and time to recurrence when compared to MSS [73][74]. Moreover, dMMR status was a strong negative predictor of 5-fluorouracil efficacy, making it a good marker to guide chemotherapy choice [75]. Finally, MSI-H/dMMR status increased the ability to predict response to checkpoint inhibitors [76][77]. Patients previously treated for MSI-H/dMMR mCRC experienced a 30% plateau in OS and progression-free survival (PFS) at five years in the phase II KEYNOTE-164 study [26][78].

dMMR status can be tested via IHC, and MSI status can be tested using PCR or NGS based assays. IHC is usually the test of choice because of the test's high concordance (>90%) [79], but NGS or PCR testing can be used if IHC findings are inconclusive [80][81]. There is no need to analyze both the primary and the metastatic sites since concordance is over 90% [81].

3.4. HER2 Amplification

Human epidermal growth factor receptor 2 (HER2) is a factor involved in multiple tumor types, including colorectal, breast, and gastroesophageal cancers [82]. HER2 (also known as ERBB2) codes for a transmembrane protein on the long arm of chromosome 17 and activates downstream signaling pathways by complexes with other HER proteins [83]. Cell differentiation, migration, proliferation, and apoptosis inhibition are all facilitated by HER2 [84]. IHC and fluorescence in situ (FISH) can be used to identify HER2 overexpression and amplification [85].

HER2 amplification is present in 2–5% of all CRCs [86] with higher prevalence in wild type RAS/BRAF tumors (approximately 5–14%) [87][88]. Following anti-EGFR treatments, the frequency of HER2 overexpression increases, possibly suggesting HER2′s involvement in resistance to the anti-EGFR agents [89][90].

The NCCN Guidelines recommend HER2 amplification testing in mCRC patients, but not for KRAS/NRAS or BRAF mutant tumors [28]. It is shown that in patients with wild-type RAS/BRAF mCRC, the HER2 status did not impact median PFS on therapy without an EGFR inhibitor [91]. Thus, in patients with wild-type RAS and BRAF tumors with HER2 overexpression, HER2 targeted treatment can be used as a subsequent therapy [87][92]. Additionally, previously treated patients with HER2-positive mCRC responded to trastuzumab deruxtecan, an antibody-drug conjugate, with a 45% response rate [93]. Although HER2 is a predictive biomarker of resistance to monoclonal antibodies that target EGFR [88][91][94] the current level of evidence does not support HER2 overexpression as a prognostic tool [95].

3.5. NTRK Fusions

Neurotrophic Tropomyosin Receptor Kinase (NTRK) genes encode the tropomyosin receptor kinase (TRK) proteins involved in cell homeostasis and embryonal neural development [96][97]. These genes can fuse with other genes, producing NTRK gene fusions that can lead to uncontrolled cell growth and division [98]. These can be detected with IHC, plasma cell-free DNA profiling, and tumor DNA and RNA sequencing [99]. NTRK gene fusions exist in several cancers, including CRC, where they are present in only <1% [100][101]. NTRK fusions are more common in MSI-H/dMMR CRC [102] and are connected to APC and TP53 mutations [103]. Patients with NTRK gene fusion-positive mCRC are good candidates for TRK inhibitors therapies. According to the NCCN guidelines; larotrectinib and entrectinib have received FDA approval for treating patients with NTRK gene fusion-positive metastatic, unresectable solid tumors [28].

References

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J. Clin. 2021, 71, 209–249.
2. Foster, J.H. Treatment of metastatic disease of the liver: A skeptic's view. Semin. Liver Dis. 1984, 4, 170–179.
3. Schmoll, H.J.; Van Cutsem, E.; Stein, A.E.S.M.O.; Valentini, V.; Glimelius, B.; Haustermans, K.; Nordlinger, B.; Van de Velde, C.J.; Balmana, J.; Regula, J.; et al. ESMO Consensus Guidelines for management of patients with colon and rectal cancer. a personalized approach to clinical decision making. Ann. Oncol. 2012, 23, 2479–2516.
4. Lee, H.; Palm, J.; Grimes, S.M.; Ji, H.P. The Cancer Genome Atlas Clinical Explorer: A web and mobile interface for identifying clinical-genomic driver associations. Genome Med. 2015, 7, 112.
5. Collins, F.S.; Barker, A.D. Mapping the cancer genome. Pinpointing the genes involved in cancer will help chart a new course across the complex landscape of human malignancies. Sci. Am. 2007, 296, 50–57.
6. Hardiman, K.M. Update on Sporadic Colorectal Cancer Genetics. Clin. Colon. Rectal. Surg. 2018, 31, 147–152.
7. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012, 487, 330–337.
8. Zhan, T.; Rindtorff, N.; Boutros, M. Wnt signaling in cancer. Oncogene 2017, 36, 1461–1473.
9. Novellasdemunt, L.; Antas, P.; Li, V.S. Targeting Wnt signaling in colorectal cancer. A Review in the Theme: Cell Signaling: Proteins, Pathways and Mechanisms. Am. J. Physiol. Cell Physiol. 2015, 309, C511–C521.
10. Missiaglia, E.; Jacobs, B.; D'Ario, G.; Di Narzo, A.; Soneson, C.; Budinska, E.; Popovici, V.; Vecchione, L.; Gerster, S.; Yan, P.; et al. Distal and proximal colon cancers differ in terms of molecular, pathological, and clinical features. Ann. Oncol. 2014, 25, 1995–2001.
11. Gonzalez, E.C.; Roetzheim, R.G.; Ferrante, J.M.; Campbell, R. Predictors of proximal vs. distal colorectal cancers. Dis. Colon. Rectum. 2001, 44, 251–258.
12. Engstrand, J.; Nilsson, H.; Strömberg, C.; Jonas, E.; Freedman, J. Colorectal cancer liver metastases—A population-based study on incidence, management and survival. BMC Cancer 2018, 18, 78.
13. Tran, B.; Kopetz, S.; Tie, J.; Gibbs, P.; Jiang, Z.Q.; Lieu, C.H.; Agarwal, A.; Maru, D.; Sieber, O.; Desai, J. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. Cancer 2011, 117, 4623–4632.

14. Chen, D.; Huang, J.F.; Liu, K.; Zhang, L.Q.; Yang, Z.; Chuai, Z.R.; Wang, Y.; Shi, D.; Huang, Q.; Fu, W. BRAFV600E mutation and its association with clinicopathological features of colorectal cancer: A systematic review and meta-analysis. PLoS ONE 2014, 9, e90607.
15. Lee, M.S.; Menter, D.G.; Kopetz, S. Right Versus Left Colon Cancer Biology: Integrating the Consensus Molecular Subtypes. J. Natl. Compr. Canc. Netw. 2017, 15, 411–419.
16. Armstrong, S.A.; Malley, R.; Weinberg, B.A. Molecular Profiling in Metastatic Colorectal Cancer. Oncol. (Williston Park) 2020, 34, 352–355.
17. Yamashita, S.; Brudvik, K.W.; Kopetz, S.E.; Maru, D.; Clarke, C.N.; Passot, G.; Claudius, C.; Yun Shin, C.; Thomas, A.; Jean-Nicolas, V. Embryonic Origin of Primary Colon Cancer Predicts Pathologic Response and Survival in Patients Undergoing Resection for Colon Cancer Liver Metastases. Ann. Surg. 2018, 267, 514–520.
18. Pitroda, S.P.; Khodarev, N.N.; Huang, L.; Uppal, A.; Wightman, S.C.; Ganai, S.; Joseph, N.; Pitt, J.; Brown, M.; Forde, M.; et al. Integrated molecular subtyping defines a curable oligometastatic state in colorectal liver metastasis. Nat. Commun. 2018, 9, 1793.
19. Zhou, H.; Liu, Z.; Wang, Y.; Wen, X.; Amador, E.H.; Yuan, L.; Ran, X.; Xiong, L.; Ran, Y.; Chen, W.; et al. Colorectal liver metastasis: Molecular mechanism and interventional therapy. Signal Transduct. Target Ther. 2022, 7, 70.
20. Lavin, P.; Mittelman, A.; Douglass, H., Jr.; Engstrom, P.; Klaassen, D. Survival and response to chemotherapy for advanced colorectal adenocarcinoma: An Eastern Cooperative Oncology Group report. Cancer 1980, 46, 1536–1543.
21. Venook, A.P.; Niedzwiecki, D.; Lenz, H.J.; Innocenti, F.; Fruth, B.; Meyerhardt, J.A.; Schrag, D.; Greene, C.; O'Neil, B.H.; Atkins, J.N.; et al. Effect of First-Line Chemotherapy Combined with Cetuximab or Bevacizumab on Overall Survival in Patients With KRAS Wild-Type Advanced or Metastatic Colorectal Cancer: A Randomized Clinical Trial. JAMA 2017, 317, 2392–2401.
22. Berger, M.D.; Lenz, H.J. The safety of monoclonal antibodies for treatment of colorectal cancer. Expert Opin. Drug Saf. 2016, 15, 799–808.
23. Grothey, A.; Van Cutsem, E.; Sobrero, A.; Siena, S.; Falcone, A.; Ychou, M.; Humblet, Y.; Bouché, O.; Mineur, L.; Barone, C.; et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): An international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet 2013, 381, 303–312.
24. Mayer, R.J.; Van Cutsem, E.; Falcone, A.; Yoshino, T.; Garcia-Carbonero, R.; Mizunuma, N.; Yamazaki, K.; Shimada, Y.; Tabernero, J.; Komatsu, Y.; et al. Randomized trial of TAS-102 for refractory metastatic colorectal cancer. N. Engl. J. Med. 2015, 372, 1909–1919.
25. Holch, J.W.; Ricard, I.; Stintzing, S.; Modest, D.P.; Heinemann, V. The relevance of primary tumour location in patients with metastatic colorectal cancer: A meta-analysis of first-line clinical

trials. Eur. J. Cancer 2017, 70, 87–98.

26. Puccini, A.; Seeber, A.; Berger, M.D. Biomarkers in Metastatic Colorectal Cancer: Status Quo and Future Perspective. Cancers 2022, 14, 4828.
27. Goldstein, M.J.; Mitchell, E.P. Carcinoembryonic antigen in the staging and follow-up of patients with colorectal cancer. Cancer Investig. 2005, 23, 338–351.
28. Benson, A.B.; Venook, A.P.; Al-Hawary, M.M.; Arain, M.A.; Chen, Y.J.; Ciombor, K.K.; Cohen, S.; Cooper, H.S.; Deming, D.; Farkas, L.; et al. Colon Cancer, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J. Natl. Compr. Canc. Netw. 2021, 19, 329–359.
29. Wu, X.; Li, Z.; Huang, N.; Li, X.; Chen, R. Study of KRAS-Related miRNA Expression in Colorectal Cancer. Cancer Manag. Res. 2022, 14, 2987–3008.
30. Fernández-Medarde, A.; Santos, E. Ras in cancer and developmental diseases. Genes Cancer 2011, 2, 344–358.
31. Stangl, R.; Altendorf-Hofmann, A.; Charnley, R.M.; Scheele, J. Factors influencing the natural history of colorectal liver metastases. Lancet 1994, 343, 1405–1410.
32. Amado, R.G.; Wolf, M.; Peeters, M.; Van Cutsem, E.; Siena, S.; Freeman, D.J.; Juan, T.; Sikorski, R.; Suggs, S.; Radinsky, R.; et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J. Clin. Oncol. 2008, 26, 1626–1634.
33. Baselga, J.; Rosen, N. Determinants of RASistance to anti-epidermal growth factor receptor agents. J. Clin. Oncol. 2008, 26, 1582–1584.
34. De Roock, W.; Piessevaux, H.; De Schutter, J.; Janssens, M.; De Hertogh, G.; Personeni, N.; Biesmans, B.; Laethem, L.V.; Peeters, M.; Humblet, Y.; et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Ann. Oncol. 2008, 19, 508–515.
35. Karapetis, C.S.; Khambata-Ford, S.; Jonker, D.J.; O'Callaghan, C.J.; Tu, D.; Tebbutt, N.C.; Simes, R.J.; Chalchal, H.; Shapiro, J.D.; Robitaille, S.; et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N. Engl. J. Med. 2008, 359, 1757–1765.
36. Khambata-Ford, S.; Garrett, C.R.; Meropol, N.J.; Basik, M.; Harbison, C.T.; Wu, S.; Wong, T.W.; Huang, X.; Takimoto, C.H.; Godwin, A.K.; et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J. Clin. Oncol. 2007, 25, 3230–3237.
37. Sorich, M.J.; Wiese, M.D.; Rowland, A.; Kichenadasse, G.; McKinnon, R.A.; Karapetis, C.S. Extended RAS mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: A meta-analysis of randomized, controlled trials. Ann. Oncol. 2015, 26, 13–21.

38. Adam, R.; Delvart, V.; Pascal, G.; Valeanu, A.; Castaing, D.; Azoulay, D.; Giacchetti, S.; Paule, B.; Kunstlinger, F.; Ghémard, O.; et al. Rescue surgery for unresectable colorectal liver metastases downstaged by chemotherapy: A model to predict long-term survival. Ann. Surg. 2004, 240, 644–657; discussion 57–58.

39. Choti, M.A.; Sitzmann, J.V.; Tiburi, M.F.; Sumetchotimetha, W.; Rangsin, R.; Schulick, R.D.; Lillemoe, K.D.; Yeo, C.J.; Cameron, J.L. Trends in long-term survival following liver resection for hepatic colorectal metastases. Ann. Surg. 2002, 235, 759–766.

40. Elias, D.; Liberale, G.; Vernerey, D.; Pocard, M.; Ducreux, M.; Boige, V.; Malka, D.; Pignon, J.P.; Lasser, P. Hepatic and extrahepatic colorectal metastases: When resectable, their localization does not matter, but their total number has a prognostic effect. Ann. Surg. Oncol. 2005, 12, 900–909.

41. Fong, Y.; Salo, J. Surgical therapy of hepatic colorectal metastasis. Semin. Oncol. 1999, 26, 514–523.

42. Effendi-Ys, R. Colonoscopy, Biomarkers, and Targeted Therapy in Colorectal Cancer. Acta Med. Indones. 2022, 54, 476–486.

43. Knijn, N.; Mekenkamp, L.J.; Klomp, M.; Vink-Börger, M.E.; Tol, J.; Teerenstra, S.; Meijer, J.; Tebar, M.; Riemersma, S.; Krieken, J.H.; et al. KRAS mutation analysis: A comparison between primary tumours and matched liver metastases in 305 colorectal cancer patients. Br. J. Cancer 2011, 104, 1020–1026.

44. Sepulveda, A.R.; Hamilton, S.R.; Allegra, C.J.; Grody, W.; Cushman-Vokoun, A.M.; Funkhouser, W.K.; Kopetz, S.E.; Lieu, C.; Lindor, N.M.; Minsky, B.D.; et al. Molecular Biomarkers for the Evaluation of Colorectal Cancer: Guideline from the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology. J. Mol. Diagn. 2017, 19, 187–225.

45. Monzon, F.A.; Ogino, S.; Hammond, M.E.; Halling, K.C.; Bloom, K.J.; Nikiforova, M.N. The role of KRAS mutation testing in the management of patients with metastatic colorectal cancer. Arch. Pathol. Lab. Med. 2009, 133, 1600–1606.

46. Allegra, C.J.; Rumble, R.B.; Hamilton, S.R.; Mangu, P.B.; Roach, N.; Hantel, A.; Schilsky, R.L. Extended RAS Gene Mutation Testing in Metastatic Colorectal Carcinoma to Predict Response to Anti-Epidermal Growth Factor Receptor Monoclonal Antibody Therapy: American Society of Clinical Oncology Provisional Clinical Opinion Update 2015. J. Clin. Oncol. 2016, 34, 179–185.

47. Artale, S.; Sartore-Bianchi, A.; Veronese, S.M.; Gambi, V.; Sarnataro, C.S.; Gambacorta, M.; Lauricella, C.; Siena, S.l. Mutations of KRAS and BRAF in primary and matched metastatic sites of colorectal cancer. J. Clin. Oncol. 2008, 26, 4217–4219.

48. Etienne-Grimaldi, M.C.; Formento, J.L.; Francoual, M.; François, E.; Formento, P.; Renée, N.; Laurent-Puig, P.; Chazal, M.; Benchimol, D.; Delpero, J.R.; et al. K-Ras mutations and treatment outcome in colorectal cancer patients receiving exclusive fluoropyrimidine therapy. Clin. Cancer Res. 2008, 14, 4830–4835.
49. Davies, H.; Bignell, G.R.; Cox, C.; Stephens, P.; Edkins, S.; Clegg, S.; Teague, J.; Woffendin, H.; Garnett, M.J.; Bottomley, W.; et al. Mutations of the BRAF gene in human cancer. Nature 2002, 417, 949–954.
50. Kolch, W. Meaningful relationships: The regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. Biochem. J. 2000, 351 Pt 2, 289–305.
51. Tol, J.; Nagtegaal, I.D.; Punt, C.J. BRAF mutation in metastatic colorectal cancer. N. Engl. J. Med. 2009, 361, 98–99.
52. Peyssonnaux, C.; Eychène, A. The Raf/MEK/ERK pathway: New concepts of activation. Biol. Cell 2001, 93, 53–62.
53. Fanelli, G.N.; Pozzo, C.A.D.; Depetris, I.; Schirripa, M.; Brignola, S.; Biason, P.; Balistreri, M.; Santo, L.D.; Lonardi, S.; Munari, G.; et al. The heterogeneous clinical and pathological landscapes of metastatic Braf-mutated colorectal cancer. Cancer Cell Int. 2020, 20, 30.
54. Van Cutsem, E.; Köhne, C.H.; Láng, I.; Folprecht, G.; Nowacki, M.P.; Cascinu, S.; Balistreri, M.; Santo, L.D.; Lonardi, S.; Munari, G.; et al. 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.
55. Maughan, T.S.; Adams, R.A.; Smith, C.G.; Meade, A.M.; Seymour, M.T.; Wilson, R.H.; Idziaszczyk, S.; Harris, R.; Fisher, D.; Kenny, S.L.; et al. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: Results of the randomised phase 3 MRC COIN trial. Lancet 2011, 377, 2103–2114.
56. Tveit, K.M.; Guren, T.; Glimelius, B.; Pfeiffer, P.; Sorbye, H.; Pyrhonen, S.; Sigurdsson, F.; Kure, E.; Ikdahl, T.; Skovlund, E.; et al. Phase III trial of cetuximab with continuous or intermittent fluorouracil, leucovorin, and oxaliplatin (Nordic FLOX) versus FLOX alone in first-line treatment of metastatic colorectal cancer: The NORDIC-VII study. J. Clin. Oncol. 2012, 30, 1755–1762.
57. Wan, P.T.; Garnett, M.J.; Roe, S.M.; Lee, S.; Niculescu-Duvaz, D.; Good, V.M.; Jones, C.M.; Marshall, C.J.; Springer, C.J.; Barford, D.; et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell 2004, 116, 855–867.
58. Ikenoue, T.; Hikiba, Y.; Kanai, F.; Tanaka, Y.; Imamura, J.; Imamura, T.; Ohta, M.; Ijichi, H.; Tateishi, K.; Kawakami, T.; et al. Functional analysis of mutations within the kinase activation segment of B-Raf in human colorectal tumors. Cancer Res. 2003, 63, 8132–8137.

59. Sinicrope, F.A.; Shi, Q.; Allegra, C.J.; Smyrk, T.C.; Thibodeau, S.N.; Goldberg, R.M.; Meyers, J.P.; Pogue-Geile, K.L.; Yothers, G.; Sargent, D.; et al. Association of DNA Mismatch Repair and Mutations in BRAF and KRAS with Survival After Recurrence in Stage III Colon Cancers: A Secondary Analysis of 2 Randomized Clinical Trials. JAMA Oncol. 2017, 3, 472–480.
60. Clarke, C.N.; Kopetz, E.S. BRAF mutant colorectal cancer as a distinct subset of colorectal cancer: Clinical characteristics, clinical behavior, and response to targeted therapies. J. Gastrointest. Oncol. 2015, 6, 660–667.
61. Goel, A.; Boland, C.R. Epigenetics of colorectal cancer. Gastroenterology 2012, 143, 1442–1460.e1.
62. Leclerc, J.; Vermaut, C.; Buisine, M.P. Diagnosis of Lynch Syndrome and Strategies to Distinguish Lynch-Related Tumors from Sporadic MSI/dMMR Tumors. Cancers 2021, 13, 467.
63. Van Cutsem, E.; Cervantes, A.; Adam, R.; Sobrero, A.; Van Krieken, J.H.; Aderka, D.; Aguilar, E.A.; Bardelli, A.; Benson, A.G.; Bodoky, G.; et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann. Oncol. 2016, 27, 1386–1422.
64. Kawakami, H.; Zaanan, A.; Sinicrope, F.A. Microsatellite instability testing and its role in the management of colorectal cancer. Curr. Treat. Options Oncol. 2015, 16, 30.
65. Puccini, A.; Berger, M.D.; Zhang, W.; Lenz, H.J. What We Know About Stage II and III Colon Cancer: It's Still Not Enough. Target. Oncol. 2017, 12, 265–275.
66. Lothe, R.A.; Peltomäki, P.; Meling, G.I.; Aaltonen, L.A.; Nyström-Lahti, M.; Pylkkänen, L.; Heimdal, K.; Andersen, T.I.; Møller, P.; Rognum, T.O.; et al. Genomic instability in colorectal cancer: Relationship to clinicopathological variables and family history. Cancer Res. 1993, 53, 5849–5852.
67. Kane, M.F.; Loda, M.; Gaida, G.M.; Lipman, J.; Mishra, R.; Goldman, H.; Jessup, J.M.; Kolodner, R. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. Cancer Res. 1997, 57, 808–811.
68. Cunningham, J.M.; Kim, C.Y.; Christensen, E.R.; Tester, D.J.; Parc, Y.; Burgart, L.J.; Halling, K.C.; McDonnell, S.K.; Schaid, D.J.; Vockley, C.W.; et al. The frequency of hereditary defective mismatch repair in a prospective series of unselected colorectal carcinomas. Am. J. Hum. Genet. 2001, 69, 780–790.
69. Topalian, S.L.; Hodi, F.S.; Brahmer, J.R.; Gettinger, S.N.; Smith, D.C.; McDermott, D.F.; Powderly, J.D.; Carvajal, R.D.; Sosman, J.A.; Atkins, M.B.; et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N. Engl. J. Med. 2012, 366, 2443–2454.
70. Cercek, A.; Lumish, M.; Sinopoli, J.; Weiss, J.; Shia, J.; Lamendola-Essel, M.; El Dika, I.H.; Segal, N.; Shcherba, M.; Sugarman, R.; et al. PD-1 Blockade in Mismatch Repair-Deficient, Locally

Advanced Rectal Cancer. N. Engl. J. Med. 2022, 386, 2363–2376.

71. Sui, Q.; Zhang, X.; Chen, C.; Tang, J.; Yu, J.; Li, W.; Han, K.; Jiang, W.; Liao, L.; Kong, L.; et al. Inflammation promotes resistance to immune checkpoint inhibitors in high microsatellite instability colorectal cancer. Nat. Commun. 2022, 13, 7316.
72. Sinicrope, F.A. Lynch Syndrome-Associated Colorectal Cancer. N. Engl. J. Med. 2018, 379, 764–773.
73. Taieb, J.; Karoui, M.; Basile, D. How I treat stage II colon cancer patients. ESMO Open 2021, 6, 100184.
74. Koenig, J.L.; Toesca, D.A.S.; Harris, J.P.; Tsai, C.J.; Haraldsdottir, S.; Lin, A.Y.; Pollom, E.L.; Chang, D.T. Microsatellite Instability and Adjuvant Chemotherapy in Stage II Colon Cancer. Am. J. Clin. Oncol. 2019, 42, 573–580.
75. Sargent, D.J.; Marsoni, S.; Monges, G.; Thibodeau, S.N.; Labianca, R.; Hamilton, S.R.; French, A.J.; Kabat, B.; Foster, N.R.; Torri, V.; 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, 3219–3226.
76. Le, D.T.; Uram, J.N.; Wang, H.; Bartlett, B.R.; Kemberling, H.; Eyring, A.D.; Skora, A.D.; Luber, B.S.; Azad, N.S.; Laheru, D.; et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N. Engl. J. Med. 2015, 372, 2509–2520.
77. Le, D.T.; Durham, J.N.; Smith, K.N.; Wang, H.; Bartlett, B.R.; Aulakh, L.K.; Lu, S.; Kemberling, H.; Wilt, C.; Luber, B.S.; et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 2017, 357, 409–413.
78. Le, D.T.; Kim, T.W.; Van Cutsem, E.; Geva, R.; Jäger, D.; Hara, H.; Burge, M.; O'Neil, B.; Kavan, P.; Yoshino, T.; et al. Phase II Open-Label Study of Pembrolizumab in Treatment-Refractory, Microsatellite Instability-High/Mismatch Repair-Deficient Metastatic Colorectal Cancer: KEYNOTE-164. J. Clin. Oncol. 2020, 38, 11–19.
79. De Salins, A.G.D.A.; Tachon, G.; Cohen, R.; Karayan-Tapon, L.; Junca, A.; Frouin, E.; Godet, J.; Evrard, C.; Randrian, V.; Duval, A.; et al. Discordance between immunochemistry of mismatch repair proteins and molecular testing of microsatellite instability in colorectal cancer. ESMO Open 2021, 6, 100120.
80. Luchini, C.; Bibeau, F.; Ligtenberg, M.J.L.; Singh, N.; Nottegar, A.; Bosse, T.; Miller, R.; Riaz, N.; Douillard, J.Y.; Andre, F.; et al. ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: A systematic review-based approach. Ann. Oncol. 2019, 30, 1232–1243.
81. Martelli, V.; Pastorino, A.; Sobrero, A.F. Prognostic and predictive molecular biomarkers in advanced colorectal cancer. Pharmacol. Ther. 2022, 236, 108239.

82. Li, Z.; Chen, S.; Feng, W.; Luo, Y.; Lai, H.; Li, Q.; Xiu, B.; Li, Y.; Li, Y.; Huang, S.; et al. A pan-cancer analysis of HER2 index revealed transcriptional pattern for precise selection of HER2-targeted therapy. EBioMedicine 2020, 62, 103074.
83. Rau, A.; Janssen, N.; Kühl, L.; Sell, T.; Kalmykova, S.; Mürdter, T.E.; Dahlke, M.H.; Sers, C.; Morkel, M.; Schwab, M.; et al. Triple Targeting of HER Receptors Overcomes Heregulin-mediated Resistance to EGFR Blockade in Colorectal Cancer. Mol. Cancer Ther. 2022, 21, 799–809.
84. Graus-Porta, D.; Beerli, R.R.; Hynes, N.E. Single-chain antibody-mediated intracellular retention of ErbB-2 impairs Neu differentiation factor and epidermal growth factor signaling. Mol. Cell Biol. 1995, 15, 1182–1191.
85. Menter, D.G.; Davis, J.S.; Broom, B.M.; Overman, M.J.; Morris, J.; Kopetz, S. Back to the Colorectal Cancer Consensus Molecular Subtype Future. Curr. Gastroenterol. Rep. 2019, 21, 5.
86. Siena, S.; Sartore-Bianchi, A.; Marsoni, S.; Hurwitz, H.I.; McCall, S.J.; Penault-Llorca, F.; Srock, S.; Bardelli, A.; Trusolino, L. Targeting the human epidermal growth factor receptor 2 (HER2) oncogene in colorectal cancer. Ann. Oncol. 2018, 29, 1108–1119.
87. Sartore-Bianchi, A.; Trusolino, L.; Martino, C.; Bencardino, K.; Lonardi, S.; Bergamo, F.; Zagonel, V.; Leone, F.; Depetris, I.; Martinelli, E.; et al. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): A proof-of-concept, multicentre, open-label, phase 2 trial. Lancet Oncol. 2016, 17, 738–746.
88. Sartore-Bianchi, A.; Amatu, A.; Porcu, L.; Ghezzi, S.; Lonardi, S.; Leone, F.; Bergamo, F.; Fenocchio, E.; Martinelli, E.; Borelli, B.; et al. HER2 Positivity Predicts Unresponsiveness to EGFR-Targeted Treatment in Metastatic Colorectal Cancer. Oncologist 2019, 24, 1395–1402.
89. Bertotti, A.; Migliardi, G.; Galimi, F.; Sassi, F.; Torti, D.; Isella, C.; Corà, D.; Nicolantonio, F.D.; Buscarino, M.; Petti, C.; et al. A molecularly annotated platform of patient-derived xenografts (“xenopatients”) identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov. 2011, 1, 508–523.
90. Yonesaka, K.; Zejnullahu, K.; Okamoto, I.; Satoh, T.; Cappuzzo, F.; Souglakos, J.; Ercan, D.; Rogers, A.; Roncalli, M.; Takeda, M.; et al. Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab. Sci. Transl. Med. 2011, 3, 99ra86.
91. Raghav, K.; Loree, J.M.; Morris, J.S.; Overman, M.J.; Yu, R.; Meric-Bernstam, F.; Menter, D.; Korphaisarn, K.; Kee, B.; Muranyi, A.; et al. Validation of HER2 Amplification as a Predictive Biomarker for Anti-Epidermal Growth Factor Receptor Antibody Therapy in Metastatic Colorectal Cancer. JCO Precis. Oncol. 2019, 3, 1–13.
92. Hainsworth, J.D.; Meric-Bernstam, F.; Swanton, C.; Hurwitz, H.; Spigel, D.R.; Sweeney, C.; Burris, H.; Bose, R.; Yoo, B.; Stein, A.; et al. Targeted Therapy for Advanced Solid Tumors on the Basis

of Molecular Profiles: Results from MyPathway, an Open-Label, Phase IIa Multiple Basket Study. J. Clin. Oncol. 2018, 36, 536–542.

93. Siena, S.; Di Bartolomeo, M.; Raghav, K.; Masuishi, T.; Loupakis, F.; Kawakami, H.; Yamaguchi, K.; Nishina, T.; Fakih, M.; Elez, E.; et al. Trastuzumab deruxtecan (DS-8201) in patients with HER2-expressing metastatic colorectal cancer (DESTINY-CRC01): A multicentre, open-label, phase 2 trial. Lancet Oncol. 2021, 22, 779–789.
94. Martin, V.; Landi, L.; Molinari, F.; Fountzilas, G.; Geva, R.; Riva, A.; Saletti, P.; Dosso, S.D.; Spitale, A.; Tejpar, S.; et al. HER2 gene copy number status may influence clinical efficacy to anti-EGFR monoclonal antibodies in metastatic colorectal cancer patients. Br. J. Cancer 2013, 108, 668–675.
95. Wu, S.W.; Ma, C.C.; Li, W.H. Does overexpression of HER-2 correlate with clinicopathological characteristics and prognosis in colorectal cancer? Evidence from a meta-analysis. Diagn. Pathol. 2015, 10, 144.
96. Nakagawara, A. Trk receptor tyrosine kinases: A bridge between cancer and neural development. Cancer Lett. 2001, 169, 107–114.
97. Chao, M.V. Neurotrophins and their receptors: A convergence point for many signalling pathways. Nat. Rev. Neurosci. 2003, 4, 299–309.
98. Drilon, A.; Laetsch, T.W.; Kummar, S.; DuBois, S.G.; Lassen, U.N.; Demetri, G.D.; Nathenson, M.; Doebele, R.C.; Farago, A.F.; Pappo, A.S.; et al. Efficacy of Larotrectinib in TRK Fusion-Positive Cancers in Adults and Children. N. Engl. J. Med. 2018, 378, 731–739.
99. Cocco, E.; Scaltriti, M.; Drilon, A. NTRK fusion-positive cancers and TRK inhibitor therapy. Nat. Rev. Clin. Oncol. 2018, 15, 731–747.
100. Gatalica, Z.; Xiu, J.; Swensen, J.; Vranic, S. Molecular characterization of cancers with NTRK gene fusions. Mod. Pathol. 2019, 32, 147–153.
101. Okamura, R.; Boichard, A.; Kato, S.; Sicklick, J.K.; Bazhenova, L.; Kurzrock, R. Analysis of NTRK Alterations in Pan-Cancer Adult and Pediatric Malignancies: Implications for NTRK-Targeted Therapeutics. JCO Precis. Oncol. 2018, 2018, 1–20.
102. Guo, Y.; Guo, X.L.; Wang, S.; Chen, X.; Shi, J.; Wang, J.; Wang, K.; Klempner, S.J.; Wang, W.; Xiao, M.; et al. Genomic Alterations of NTRK, POLE, ERBB2, and Microsatellite Instability Status in Chinese Patients with Colorectal Cancer. Oncologist 2020, 25, e1671–e1680.
103. Wang, H.; Li, Z.W.; Ou, Q.; Wu, X.; Nagasaka, M.; Shao, Y.; Ou, I.; Yang, Y. NTRK fusion positive colorectal cancer is a unique subset of CRC with high TMB and microsatellite instability. Cancer Med. 2022, 11, 2541–2549.