review article

Predictive Biomarkers in Metastatic Colorectal Cancer: A Systematic Review

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abstract

PURPOSE The development and use of predictive biomarkers to guide treatment decisions are paramount not only for improving survival in patients with metastatic colorectal cancer (mCRC), but also for sparing them from unnecessary toxicity and reducing the economic burden of expensive treatments. We conducted a systematic review of published studies and evaluated the predictive biomarker landscape in the mCRC setting from a molecular and clinical viewpoint.

METHODS Studies analyzing predictive biomarkers for approved therapies in patients with mCRC were identified systematically using electronic databases. Preclinical studies and those providing no relevant information were excluded.

RESULTS A total of 173 studies comprising 148 biomarkers were selected for final analysis. Of all the biomarkers analyzed, 1.4% (two of 148) were explored in a prospective manner, whereas 98.6% (146 of 148) were evaluated in retrospective studies. Of the latter group, 78.8% (115 of 146) were not tested in subsequent phases, 9.6% (14 of 146) were tested in other retrospective cohorts, 8.9% (13 of 146) were retrospectively tested in at least one or more randomized cohorts, and only 2.7% (four of 146) were prospectively tested in a clinical trial. Finally, only 1.4% (two of 148) were validated sufficiently and are recognized as biomarkers for guiding treatment decision making in patients with mCRC. These markers were *RAS* mutational status for anti-EGFR antibodies and microsatellite instability status for anti–programmed cell death-1 drugs.

CONCLUSION Despite notable efforts to identify predictive biomarkers for various therapies used in the mCRC setting, because of a lack of data beyond retrospective studies and successful biomarker-driven approaches, only two molecular biomarkers have thus far found their translation into the clinic, highlighting the imperative need for implementing novel strategies and additional research in this clinically important field.

INTRODUCTION

Colorectal cancer (CRC) remains the third leading cause of cancer-related deaths in the western world. Despite ongoing efforts aimed at increased population screening and improved early detection strategies, approximately 20% of patients still present with metastatic disease at diagnosis, and approximately 35% of those who undergo curative surgeries for a localized disease relapse.[1] During the past three decades, the median overall survival (OS) of patients with metastatic CRC (mCRC) has gradually increased because of the implementation of combined chemotherapy regimens as well as targeted molecular therapies against EGFR and angiogenic factors.[2] Since the identification of *RAS* mutations as a negative predictive marker, anti-EGFR therapy has had the greatest impact on the management of patients with mCRC; nonetheless, the response rates of these treatments remain only approximately 40% to 60%. In addition, the recognition of immunotherapy in the treatment landscape for patients with microsatellite instability-high (MSI-H) or DNA deficient mismatch-repair (dMMR) mCRC has been encouraging for this subset of patients. In this review focused on mCRC, we systematically summarize the most relevant milestones achieved in the field of predictive biomarkers for various treatments and discuss methodologic aspects, current trends, and future directions in this exciting area.

METHODS

A systematic literature search was conducted using PubMed, EMBASE, and Web of Science up to January 2018. The query was developed and executed in PubMed (Appendix Fig A1) and was subsequently translated to other databases. For all selected articles, titles and abstracts were examined to exclude review articles and studies lacking evidence-based data. All remaining articles were screened carefully, and the bibliographies from these publications were also screened for other relevant studies. Duplicate studies

ASSOCIATED CONTENT

Appendix

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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or articles that did not meet these criteria on full review were subsequently excluded. The most pertinent articles were selected for inclusion in this review (Fig 1). The results were reviewed by a multidisciplinary team of medical oncologists and translational research scientists with longstanding expertise in this biomarker field. Critical issues regarding the biomarker study design were identified (Data Supplement), and key findings from the selected studies are summarized succinctly in this systematic review on this important clinical topic.

RESULTS

A total of 173 studies comprising 148 biomarkers, individually or as a panel, were selected for this review article. The detailed strategy for study and biomarker selection is illustrated in Fig 2.

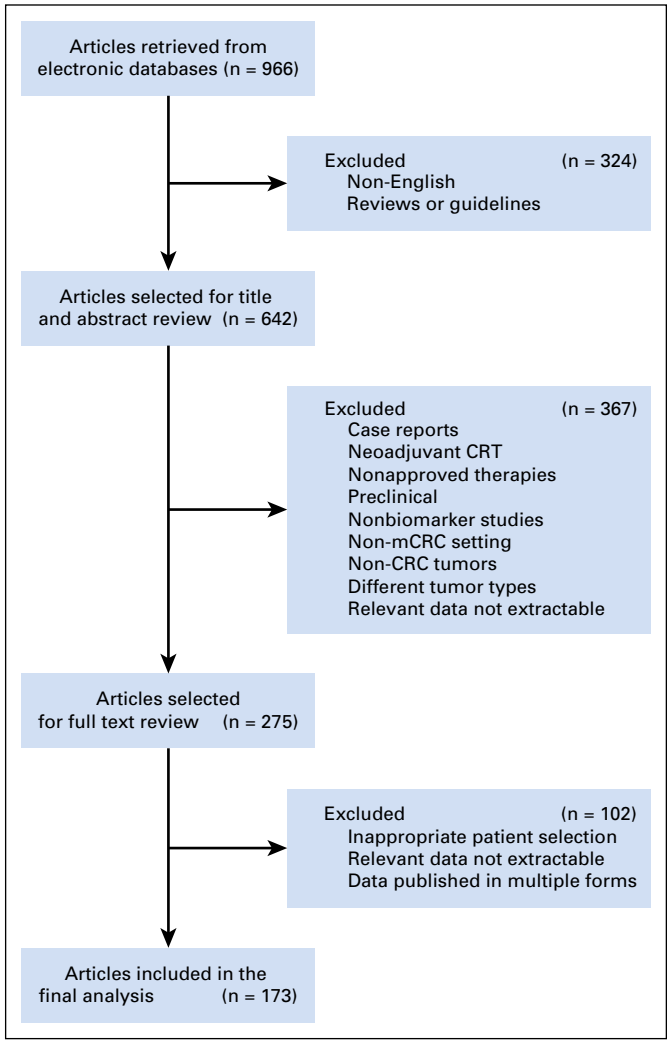

FIG 1. Flow diagram of the study selection process. CRC, colorectal cancer; mCRC, metastatic colorectal cancer; Neoadjuvant-CRT, neoadjuvant chemoradiotherapy.

Conventional Chemotherapy and Trifluridine/Tipiracil

The backbone of treatment in patients with mCRC has historically been chemotherapy, and several chemotherapeutic agents are now approved in this setting: fluoropyrimidines (fluorouracil [FU] and capecitabine), oxaliplatin, irinotecan, and since 2015, trifluridine/tipiracil (TAS-102).

Fluoropyrimidine-based chemotherapy and TAS-102. Over the years, several studies have investigated the predictive role of fluoropyrimidine metabolic pathway enzymes in response to FU-based therapies. Studies of the role of thymidylate synthase (TS) in fluoropyrimidine-based therapy (primarily FU plus leucovorin) in various retrospective and prospective studies have yielded discordant results. In this regard, multiple studies have shown that low levels of TS expression in metastatic tumor tissues generally correlate with higher overall response rate (ORR).[3-6] Surprisingly, such a correlation was not evident when TS levels were measured in primary tumor tissues.[4,7] Similarly, low levels of TS and dihydropyrimidine dehydrogenase in metastatic tumor tissues were associated with a favorable response to FU in patients with mCRC[8]; however, a subsequent report in 2006 did not validate these findings.[9] Likewise, the role of thymidine phosphorylase as a predictive biomarker was also investigated, but the results remain inconclusive.[10,11]

In 2009, a meta-analysis of five studies examining a total of 861 patients with mCRC concluded that compared with microsatellite-stable patients, MSI-H patients did not achieve a statistically significant better response rate to FU-based chemotherapy.[12] Similarly, while investigating the relationship between *MSH2* gene expression and capecitabine efficacy in patients with mCRC, Jensen et al[13] observed that a higher *MSH2* expression was associated with a better response. In an attempt to identify noncoding RNA-based predictive biomarkers, a low expression of miR-143 was shown to be associated with improved ORR and progression-free survival (PFS) in patients treated with capecitabine.[14] Likewise, a low expression of miR-429 correlated with improved 5-year disease-free survival and OS in patients with mCRC treated with FU-based chemotherapy.[15] Other predictive biomarkers for fluoropyrimidine-based chemotherapy are summarized in Table 1 and in the Data Supplement.

On the basis of the results of the phase III RECOURSE (Study of TAS-102 in Patients With Metastatic Colorectal Cancer Refractory to Standard Chemotherapies) trial, the US Food and Drug Administration approved TAS-102 for patients with mCRC. In 2015, Hamauchi et al[30] found that patients who develop grade 3 or 4 neutropenia during the first cycle of TAS-102 treatment had a smaller risk of disease progression. More recently, another study reported improved OS rates in patients harboring any G allele at the *ATM* rs609429 locus, when compared with those with a C/C variant.[16]

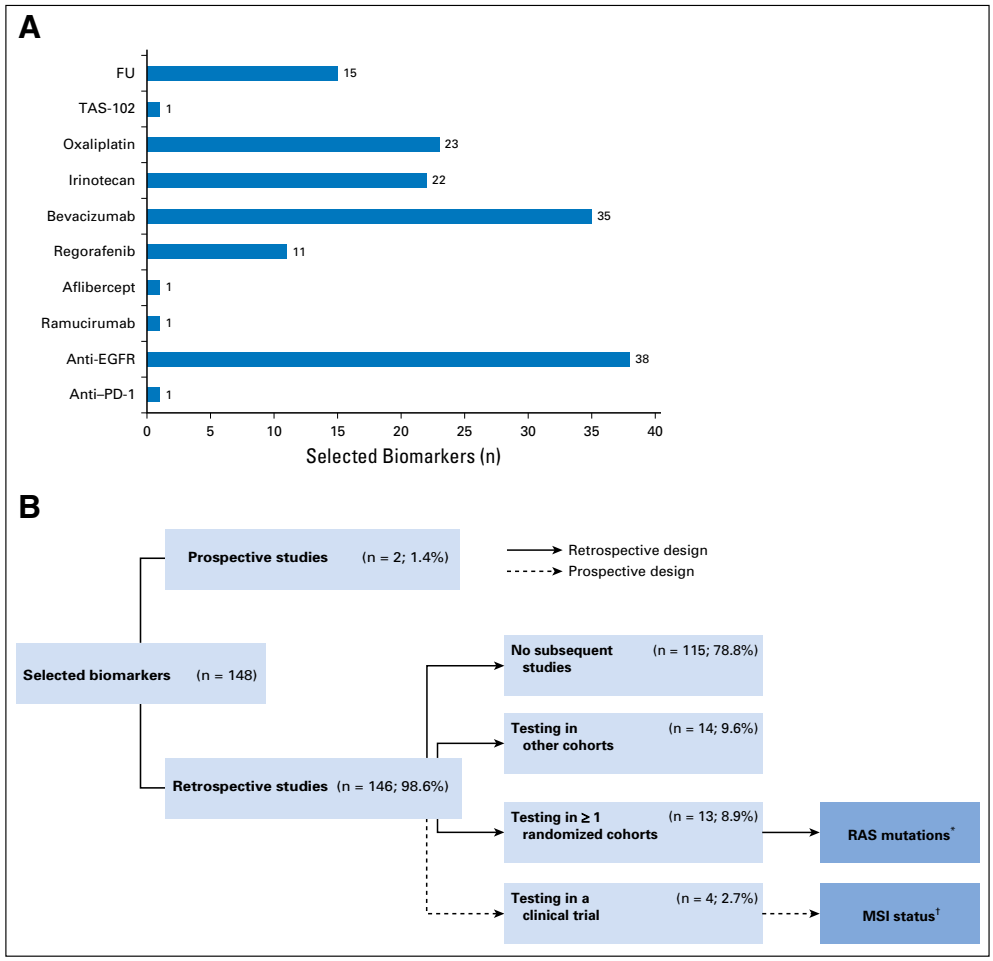

FIG 2. Selected biomarkers for the final analysis. (A) Distribution of biomarkers by individual treatment group. (B) Distribution of biomarkers by study design. Anti–PD-1, anti–programmed cell death-1 drugs; FU, fluoropyrimidine-based chemotherapy; Irinotecan, irinotecan-based chemotherapy; MSI, microsatellite instability; Oxaliplatin, oxaliplatin-based chemotherapy; TAS-102, trifluridine/tipiracil. (*) Predictive biomarker for anti-EGFR drugs (cetuximab/panitumumab); (†) predictive biomarker for anti–PD-1 drugs (pembrolizumab and nivolumab alone or in combination with ipilimumab).

Oxaliplatin-based chemotherapy. In examining the mechanism of action of oxaliplatin, several key genes involved in the nucleotide excision repair pathway have been explored as potential predictive biomarkers. The most notable attempts in this regard have been for the *ERCC1* gene, and as reported from the MAVERICC (Marker Evaluation for Avastin Research in CRC) trial, intratumoral *ERCC1* gene expression failed to predict response to oxaliplatin treatment.[17] Another gene evaluated in this setting was the x-ray repair cross-complementing group 1 (*XRCC1*) gene, a base excision repair modulator, wherein a polymorphism in this gene (*XRCC1*-839 Arg/Gln or Gln/Gln) correlated with worse ORR to FU/oxaliplatin.[18]

Interestingly, several microRNAs (miRNAs) have been explored for their predictive response potential to FU, leucovorin, and oxaliplatin (FOLFOX)– or capecitabine plus oxaliplatin (CAPEOX)–based regimens. In this treatment setting, high miR-625-3p and low miR-148a expression were associated with poor response,[19,20] whereas high miR-126 microvessel density was associated with improved PFS.[21]

In addition to studies of messenger RNA- and miRNA-based markers, two independent studies reported that evaluation of serum protein expression patterns is implicated in predicting response to CAPEOX and FOLFOX, respectively.[22,31] Other potential predictive biomarkers for oxaliplatin-based chemotherapy are summarized in Table 1 and in the Data Supplement.

Irinotecan-based chemotherapy. In terms of predictive biomarkers for irinotecan treatment, the most notable marker studied in this setting is topoisomerase 1 (TOP1). The first large study in which TOP1 predictive power was evaluated used samples from the FOCUS (Fluorouracil, Oxaliplatin, CPT-11: Use and Sequencing) trial and reported that a moderate or high expression of TOP1 was associated with a significant gain in survival after irinotecan-based therapy.[23] Unfortunately, these findings were not confirmed subsequently by analyzing samples from 545 patients involved in the CAIRO (Capecitabine, Irinotecan, Oxaliplatin) study, despite similar treatment regimens and analytic approaches.[24]

While studying the role of genetic polymorphisms within the *TDP1* and *XRCC1* genes in response to irinotecan-based regimens, a positive correlation with improved ORR was observed in patients with the *TDP1* IVS12+79G>T and *XRCC1* GGCC-G/GGCC-G genotypes.[25] Aprataxin, a protein member of the histidine triad superfamily, has also shown a potential ability to discriminate responders from

TABLE 1. Predictive Biomarkers for Response to Conventional Chemotherapy and TAS-102

Biomarker	Line of Therapy	Biomarker Type	Study Design	Specimen	Analytic Method	Sample Type	Discovery Cohort (n)	Response Criteria	Results
Fluoropyrimidine-based chemotherapy									
TS[3]	1	Proteomic	Retrospective	Metastatic tumor tissue	IHC	FFPE	41	WHO	Low expression: improved ORR, $P = .0001$*
TS[4]	1	Proteomic	Retrospective	Primary tumor tissue	IHC	FFPE	27	—	No correlation: ORR, $P = .47$
TS[4]	1	Proteomic	Retrospective	Metastatic tumor tissue	IHC	FFPE	27	—	Low expression: improved ORR, $P = .012$*
TS[5]	1	Proteomic	Retrospective	Metastatic tumor tissue	IHC	FFPE	48	WHO	Low expression: improved ORR, $P = .003$*
TS[5]	1	Proteomic	Retrospective	Metastatic tumor tissue	IHC	FFPE	41	WHO	Low expression: improved ORR, $P < .0001$*
TS[6]	1	Proteomic	Retrospective	Metastatic tumor tissue	TRA	Frozen	93	WHO	Low expression: improved ORR, $P = .047$*
TS[7]	1	Proteomic	Retrospective	Primary tumor tissue	IHC	FFPE	219	—	No correlation: ORR, $P = .084$
									No correlation: 2-year OS, $P > .1$
TS & DPD[9]	1	Gene expression	Prospective	Metastatic tumor tissue	RT-qPCR	Frozen	31	WHO	No correlation: ORR, $P = .10$
TP[10]	1	Gene expression	Retrospective	Primary tumor tissue	RT-qPCR	FFPE	144	WHO	Low expression: improved ORR, $P = .015$*
									Low expression: improved TTP, $P < .001$*
									Low expression: improved OS, $P = .002$*
TP[11]	1	Gene expression	Retrospective	Primary tumor tissue	RT-qPCR	Frozen	125	WHO	No correlation: ORR, $P =$ ns
									No correlation: OS, $P =$ ns
									High expression: improved TTP, $P < .05$*
MMR[12]	1-2	Proteomic	Meta-analysis	Tumor tissue	IHC/PCR	—	5 studies (860)	—	No correlation: ORR, $P = .11$
		Genomic							PFS, not reported
									OS, not reported
MSH2[13]	1	Gene expression	Retrospective	Primary tumor tissue	RT-qPCR	—	37	WHO	NR (lower expression) *v* R (higher expression), $P = .038$*
									No correlation: OS, $P = .083$
miR-143[14]	1	Epigenomic (miRNAs)	Retrospective	Primary tumor tissue	RT-qPCR	FFPE	55	RECIST	Low expression: improved ORR, $P = .037$*
									Low expression: improved PFS, $P = .012$*
									No correlation: OS, $P =$ ns

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TABLE 1. Predictive Biomarkers for Response to Conventional Chemotherapy and TAS-102 (Continued)

Biomarker	Line of Therapy	Biomarker Type	Study Design	Specimen	Analytic Method	Sample Type	Discovery Cohort (n)	Response Criteria	Results
miR-429[15]	1	Epigenomic (miRNAs)	Retrospective	Primary tumor tissue	RT-qPCR	Frozen	116	RECIST 1.1	NR (higher expression) *v* R (lower expression), $P < .001$*
									Low level:
									Improved 5-year survival rate, $P = .0011$*
									Improved OS, $P = .0001$*
TAS-102									
ATM rs60942[16]	> 1	Genomic (polymorphisms)	Retrospective	Blood	Sequencing	—	104 (VC, n = 129)	RECIST 1.1	No correlation: ORR, P = ns
									No correlation: PFS, P = ns
									ATM rs60942 C/C: worse OS, $P = .020$*
Oxaliplatin-based chemotherapy									
ERCC1[17]	1	Gene expression	Prospective	Tumor tissue	RT-qPCR	—	376	RECIST 1.1	No correlation: PFS, $P = .3944$
									No correlation: OS, $P = .3295$
XRCC1[18]	—	Genomic (polymorphisms)	Retrospective	Blood	PCR-RFLP	—	61	WHO	XRCC1-839 Arg/Gln or Gln/Gln: worse ORR, $P = .038$*
									No correlation: Survival, $P = 0.38$
miR-625-3p[19]	1	Epigenomic (miRNAs)	Retrospective	Primary tumor tissue	RT-qPCR	Frozen	26 (VC, n = 94)	RECIST	Low expression: improved ORR, $P = .009$*
									High expression: higher NR risk, $P = .003$*
									Low expression, no correlation: PFS, $P = .316$
									Low expression: improved OS, $P = .039$*
miR-148a[20]	1	Epigenomic (miRNAs)	Retrospective	Primary tumor tissue	RT-qPCR	FFPE	72	RECIST	High expression: improved ORR, $P = .006$*
									High expression, no correlation: PFS, $P = .16$
									High expression: improved OS, $P = .024$*
MVDi by miR-126[21]	1	Epigenomic (miRNAs)	Retrospective	Primary tumor tissue	miR-126-ISH	FFPE	89	RECIST	R (higher value) *v* NR (lower value), $P = .01$*
									High MVDi: improved PFS, $P = .01$*
									No correlation: OS, $P = .06$
m/z 3330 Da[22]	1	Proteomic	Prospective	Serum	SELDI-TOF MS	—	42	RECIST	NR (lower peak intensity) *v* R (higher peak intensity), $P < .01$*
Irinotecan-based chemotherapy									
TOP1[23]	1	Proteomic	Retrospective	Primary tumor tissue	IHC	FFPE	1,313	RECIST	Moderate/high expression: improved PFS, $P = .001$*
TOP1[24]	1	Proteomic	Retrospective	Primary tumor tissue	IHC	FFPE	545	RECIST	Moderate/high expression: no correlation: PFS, $P = .710$
									No correlation: OS, $P = .65$

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TABLE 1. Predictive Biomarkers for Response to Conventional Chemotherapy and TAS-102 (Continued)

Biomarker	Line of Therapy	Biomarker Type	Study Design	Specimen	Analytic Method	Sample Type	Discovery Cohort (n)	Response Criteria	Results
TDP1 IVS12 +79[25]	≥ 1	Genomic (polymorphisms)	Retrospective	Blood	Sequencing	—	107	—	TDP1 IVS12+79 G/G: improved ORR, P = .044*
XRCC1[25]	≥ 1	Genomic (polymorphisms)	Retrospective	Blood	Sequencing	—	107	—	XRCC1 GGCC-G/GGCC-G diplotype, improved ORR, P = .016*
Aprataxin[26]	1	Proteomic	Retrospective	Tumor tissue	IHC	FFPE	128	RECIST	Low level: improved DCR, P = .04*
									Low level: improved PFS, P = .03*
									Low level: improved OS, P = .008*
UGT1A[27]	1	Genomic (polymorphisms)	Retrospective	Blood	Pyrosequencing	—	238	WHO	UGT1A1*28/*28:
									Improved ORR, P = .02*
									Improved DCR, P = .04*
									Improved TTP, P = .02*
									2 UGT1A haplotype II alleles: improved ORR, P = .01*
UGT1A1*28[28]	≥ 1	Genomic (polymorphisms)	Meta-analysis	Not specified	Not specified	—	12 studies (1,898)	RECIST	No correlation: ORR, P = ns
								WHO	
BNIP3[29]	1-4	Epigenomic (DNA methylation)	Retrospective	Tumor tissue	qMSP	Frozen	27	RECIST	NR (higher methylation level) *v* R (lower methylation level), P = .031*

Abbreviations: Da, Dalton; DCR, disease control rate; DPD, dihydropyrimidine dehydrogenase; FFPE, formalin-fixed paraffin-embedded; IHC, immunohistochemistry; ISH, in situ hybridization; m/z, mass-to-charge ratio; miRNA, microRNA; MMR, mismatch repair; MVD, microvessel density; NR, nonresponders; ns, not statistically significant; ORR, overall response rate; OS, overall survival; PCR, polymerase chain reaction; PFS, progression-free survival; qMSP, quantitative methylation-specific polymerase chain reaction; R, responders; RECIST, Response Evaluation Criteria in Solid Tumors; RFLP, restriction fragment length polymorphism; RT-qPCR, quantitative reverse transcription polymerase chain reaction; SELDI-TOF-MS, surface-enhanced laser desorption/ionization time-of-flight mass spectrometry; TAS-102, trifluridine/tipiracil; TOP1, topoisomerase 1; TP, thymidine phosphorylase; TRA, tritium release assay; TS, thymidylate synthase; TTP, time to progression; VC, validation cohort; XRCC1, x-ray repair cross-complementing group 1.

*Statistically significant.

nonresponders. In a retrospective cohort of 128 patients treated with irinotecan-based chemotherapy, a low expression of aprataxin correlated with improved disease control rate, PFS, and OS.[26] Although studies of *UGT1A* gene polymorphisms and their predictive value for response to irinotecan treatment have yielded conflicting results,[27,28] higher methylation levels of the *BNIP3* gene in patients treated with irinotecan plus S1 correlated with lower response rates.[29] Additional predictive biomarkers for irinotecan-based chemotherapy are summarized in Table 1 and in the Data Supplement.

Antiangiogenic Drugs

Bevacizumab. Since the introduction of bevacizumab for the management of patients with mCRC, substantial efforts have been made to discover predictive biomarkers for this antiangiogenic drug. Using a proteomic approach in 2010, Aoyagi et al[32] reported that lower levels of plasma soluble vascular endothelial growth factor (VEGF) receptor 1 were associated with improved disease control in a subset of 46 patients treated with modified FU, leucovorin, and oxaliplatin (mFOLFOX6) plus bevacizumab. In the same year, another study identified a significant correlation between low angiopoetin-2 serum levels and better survival outcomes in a cohort of patients with mCRC treated with bevacizumab-based therapy.[33]

Conversely, the correlation between VEGF-A levels and the clinical benefit of bevacizumab-based chemotherapy is still under evaluation. In 2014, a study by Bruhn et al[34] reported higher ORR and improved PFS in patients with a low expression of VEGF-A in primary tumor specimens. Perhaps in the near future, MAVERICC trial results will offer more insights into the usefulness of plasma VEGF-A levels in this setting.[17] With regard to the predictive role of *RAS*, in a subset of 230 patients with mCRC treated in a phase III randomized clinical trial with either irinotecan, FU, and leucovorin (IFL) plus placebo, or IFL plus bevacizumab, only the wild-type *KRAS* subset of patients obtained a significantly higher response rate in the bevacizumab arm.[35]

In addition to the molecular markers listed previously, a few clinical factors have been studied in relation to bevacizumab response. Perhaps the best documented feature in this context is the association between bevacizumab-induced hypertension and a better outcome in terms of response rate, PFS, and OS.[36] On the contrary, another clinical factor such as a high visceral fat area has been associated with poor response rate, time to progression, and OS.[37] Other potential predictive biomarkers for bevacizumab-based therapy are summarized in Table 2 and in the Data Supplement.

Regorafenib. Two retrospective studies based on the data from the CORRECT (Patients With Metastatic Colorectal Cancer Treated With Regorafenib or Placebo After Failure of Standard Therapy) and CONSIGNA (Regorafenib in Subjects With Metastatic Colorectal Cancer Who Have Progressed After Standard Therapy) trials have evaluated the usefulness of different computed tomography scan–based parameters in predicting the clinical benefit of regorafenib therapy. One of the first studies, in 2016, reported a correlation between improved PFS and lung metastases cavitation before therapy initiation and even at week 8.[38] In this study, baseline lung metastases cavitation and changes in the sum of target lesion diameters were deemed to be predictors for improved OS in the multivariate analysis. A year later, the CORRECT trial also demonstrated a significant association between survival (PFS and OS) and several radiologic parameters such as response or stable disease in size and density of lung metastases.[39] Likewise, highlighting the use of dynamic contrast-enhanced magnetic resonance imaging, a recent study reported that a $>$ 70% decrease in KeF (the product of the median values of volume transfer constant and enhancing fraction) correlated positively with an improved disease control rate and longer PFS and OS.[40] Conversely, tissue-based molecular markers such as the downregulation of p53 and phosphorylated-proline–rich AKT substrate have been shown to correlate with higher PFS and metabolic response, respectively.[41] Regarding clinical variables and on the basis of the REBECCA (Regorafenib in Metastatic Colorectal Cancer: A Cohort Study in the Real-Life Setting) study, a prognostic score was developed that included the following several parameters independently associated with poorer OS: high Eastern Cooperative Oncology Group performance status, a shorter time from initial diagnosis of metastases, an initial regorafenib dose of $<$ 160 mg, more than three metastatic sites, liver metastases, and *KRAS* mutations.[42] Other potential biomarkers for regorafenib therapy are summarized in the Data Supplement.

Aflibercept and ramucirumab. In 2015, 87 patients with mCRC enrolled as part of the phase II AFFIRM (Study of Aflibercept and Modified FOLFOX6 as First-Line Treatment in Patients With Metastatic Colorectal Cancer) trial, who were treated with aflibercept plus mFOLFOX6, were analyzed, and it was reported that high plasma levels of interleukin-8 at baseline, together with their increase, were correlated with shorter PFS.[43] In 2017, Tabernero et al[44] described, in a translational research study based on the RAISE (Ramucirumab Versus Placebo in Combination With Second-Line FOLFIRI in Patients With Metastatic Colorectal Carcinoma That Progressed During or After First-Line Therapy With Bevacizumab, Oxaliplatin, and a Fluoropyrimidine) trial, that high VEGF-D basal levels correlated with a better PFS and OS in patients treated in the ramucirumab arm (Data Supplement).

Anti-EGFR Drugs: Cetuximab and Panitumumab

KRAS mutations in tumor tissues were the first predictive biomarker approved to guide decision making for determining eligibility for anti-EGFR therapy in patients with mCRC. One of the first studies comprising a series of 30 patients with mCRC treated with cetuximab-based

TABLE 2. Predictive Biomarkers for Response to Bevacizumab

Biomarker	Line of Therapy	Biomarker Type	Study Design	Specimen	Analytic Method	Sample Type	Discovery Cohort (n)*	Response Criteria	Results
sVEGFR-1[32]	1	Proteomic	Retrospective	Plasma	Immunoassay	—	46	RECIST	R (lower level) *v* NR (higher level), $P = .025$†
									High level: worse PFS, $P = .012$†
									High level: worse OS, $P = .021$†
Angiopoietin-2[33]	1	Proteomic	Retrospective	Serum	ELISA	—	34	RECIST	Low level: improved ORR, $P < .01$†
									Low level: improved PFS, $P < .01$†
									Low level: improved OS, $P < .05$†
VEGF-A[34]	1	Proteomic	Retrospective	Primary tumor tissue	BioPlex protein array	FFPE	196	RECIST	Low level: improved ORR, $P = .03$†
									High level: worse PFS, $P = .008$†
KRAS[35]	1	Genomic (mutations)	Retrospective	Tumor tissue	Sequencing	FFPE	230	RECIST	KRAS wt: improved ORR, $P = .006$†
Hypertension[36]	—	Clinical	Meta-analysis	—	—	—	9 studies (1,674)	—	Improved ORR, $P = .0002$†
									Improved PFS, $P < .00001$†
									Improved OS, $P < .00001$†
VFA[37]	1	Clinical	Retrospective	CT scan images	CT scan	—	120	RECIST	High VFA: worse ORR, $P = .008$†
									High VFA: worse TTP, $P = .005$†
									No correlation: OS, $P = .085$

Abbreviations: CT, computed tomography; ELISA, enzyme-linked immunosorbent assay; FFPE, formalin-fixed paraffin-embedded; NR, nonresponders; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; R, responders; RECIST, Response Evaluation Criteria in Solid Tumors; sVEGFR-1, soluble vascular endothelial growth factor receptor 1; TTP, time to progression; VEGF, vascular endothelial growth factor; VFA, visceral fat area; wt, wild-type.

*No studies included a validation cohort.

†Statistically significant.

regimens noted a significant correlation between the presence of *KRAS* mutations and the lack of response to anti-EGFR therapy,[45] an observation that was validated subsequently in a cohort of 427 patients with mCRC treated with panitumumab.[46] Similarly, the role of *NRAS* mutation status as a negative response predictor for panitumumab and cetuximab was later confirmed in various clinical trials and meta-analyses.[47-51] Interestingly, in addition to the lack of efficacy in patients with *RAS*-mutant mCRC, retrospective data analysis from two phase III clinical trials reported a detrimental effect when panitumumab or cetuximab was given in combination with FOLFOX[50,52]; such a negative effect for these drugs when given in conjunction with FU, leucovorin, and irinotecan (FOLFIRI) has not been confirmed to date.[53] In an attempt to translate these tissue-based predictive biomarkers into circulating tumor DNA (ctDNA)–based liquid biopsy assays, two studies in 2017 used an innovative beads, emulsions, amplification, and magnetics (BEAMing) assay and reported promising agreement rates of 89.7% and 93% for the mutational status of *RAS* between tissue and ctDNA.[54,55]

Regarding the role of the mutant *BRAF* gene, the presence of *V600E* mutation within this gene often reflects a poor prognosis in patients with CRC.[56] In addition, two meta-analyses reported a lack of benefit in terms of PFS, OS, and ORR when anti-EGFR therapies were combined with standard chemotherapy in the subset of patients harboring *BRAF* mutations.[57,58] Despite these early data suggesting that the presence of the *BRAF V600E* mutation may dictate a lack of response to anti-EGFR–based therapies, there is still not enough clinical evidence to consider *BRAF* mutational status as a predictive biomarker in patients with advanced disease.

In 2016, in a retrospective analysis of the CRYSTAL (Cetuximab Combined With Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer) and FIRE-3 (FOLFIRI Plus Cetuximab Versus FOLFIRI Plus Bevacizumab as First-Line Treatment for Patients With Metastatic Colorectal Cancer) trials, Tejpar et al[59] highlighted the significance of tumor location within the colorectum as a predictor of treatment response to anti-EGFR drugs. In these trials, the researchers showed substantially better ORR and a corresponding increase in PFS and OS in patients with wild-type *RAS* and left-sided tumors.[59] However, a subsequent meta-analysis of results from the PRIME (Panitumumab Randomized Trial in Combination With Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy) and CRYSTAL trials in 2017 failed to show a significant correlation between primary tumor location and ORR, PFS, or OS.[60] Nonetheless, a meta-analysis of the FIRE-3/AIO KRK0306, CALGB/SWOG 80405, and PEAK (Panitumumab Efficacy in Combination With mFOLFOX6 Against Bevacizumab Plus mFOLOFOX6 in mCRC Subjects With Wild-Type KRAS Tumors) trials still demonstrated a statistically significant benefit in terms of ORR and OS in patients with wild-type *RAS* and left-sided tumors.[60] In addition to molecular markers, the secondary effects of anti-EGFR treatments, including skin rash, xerosis, or hypomagnesemia, have been postulated as serving as early response predictors. These and other potential predictive biomarkers for anti-EGFR therapy are summarized in Table 3 and in the Data Supplement.

Anti–Programmed Cell Death-1 Drugs: Pembrolizumab and Nivolumab

In May and July of 2017, the US Food and Drug Administration approved pembrolizumab and nivolumab for the treatment of patients with MSI-H mCRC in whom the disease has progressed after treatment with fluoropyrimidine, oxaliplatin, and irinotecan therapies. Almost a year later, in July 2018, a nivolumab plus ipilimumab combined regimen was approved, which opened up three novel treatment options for patients with MSI-H or dMMR mCRC, who represent approximately 5% of all patients with mCRC.[23] The belief is that, despite their worse prognosis, a large proportion of lymphocytic infiltration and the presence of mutation-associated neoantigens[61] confer to patients with MSI-positive mCRC the clinical benefit they derive from anti–programmed cell death-1 (PD-1) therapy.[62-65] This exciting discovery has now led to universal MSI testing for the management of patients with mCRC.

Disease Monitoring by Liquid Biopsies

Recently, liquid biopsies have emerged as powerful tools for monitoring disease evolution and therapeutic response through the analysis of cell-free DNA and RNA biomarkers in bodily fluids. One of the first studies, in 1979, which reported that a gradual decrease in carcinoembryonic antigen (CEA) levels during chemotherapy was significantly associated with better survival rates, was the basis for this concept.[66] Such a correlation between CEA flare and improved PFS and OS was confirmed a few years later in a subset of 670 patients with mCRC undergoing first-line chemotherapy.[67] Although CEA is not a CRC-specific biomarker, CEA monitoring in blood, alone or in addition to CA 19-9,[68] is still performed commonly in routine clinical practice. In this context, the accuracy of CEA change in predicting disease progression has been demonstrated recently in a study involving 2,828 patients from seven first-line clinical trials.[69] In addition to this, other analyses of circulating tumor cells[70] and endothelial cells[71] in mCRC have been undertaken by several groups. In 2015, Hansen et al[72] reported that circulating levels of miRNA-126 in a subset of 68 patients with mCRC were predictive of tumor response to bevacizumab-based chemotherapy. Two years later, another study reported an association between increasing levels of vasoactive peptides and better treatment outcomes.[73] The role of ctDNA in genotyping CRCs and tracking clonal evolution during and after treatment with anti-EGFR–based schedules was first evaluated by

TABLE 3. Predictive Biomarkers for Response to Anti-EGFR Drugs

Biomarker	Anti-EGFR	Line of Therapy	Biomarker Type	Study Design	Specimen	Analytic Method	Sample Type	Discovery Cohort (n)*	Response Criteria	Results†
KRAS[45]	Cetuximab	≥ 1	Genomic (mutations)	Retrospective	Primary tumor tissue	Sequencing	—	30	RECIST	R (0% mt) *v* NR (68.4% mt), *P* = .0003†
										KRAS wt: improved OS, *P* = .016†
KRAS[46]	Panitumumab	≥ 3	Genomic (mutations)	Retrospective	Tumor tissue	RT-PCR	FFPE	427	RECIST	ORR: 0% KRAS mt *v* 17% KRAS wt
										KRAS wt: improved PFS in panitumumab group, *P* < .0001†
										No correlation: OS, *P* = ns
KRAS G13D[48]	Cetuximab	> 1	Genomic (mutations)	Retrospective	Tumor tissue	Sequencing	—	7 studies + off study (579)	RECIST	G13D *v* other KRAS mt:
										No correlation: ORR, *P* = .19
										Improved PFS, *P* = .05†
										Improved OS, *P* = .003†
										KRAS *v* wt G13D:
										Improved ORR, *P* = .02†
										No correlation: PFS, *P* = .79
										No correlation: OS, *P* = .06
NRAS[49]	Cetuximab	≥ 2	Genomic (mutations)	Retrospective	Tumor tissue	Multiplex-PCR	Frozen	279 - 294	WHO	NRAS wt: improved ORR, *P* = .0040†
							FFPE		RECIST	No correlation: PFS, *P* = .068
										NRAS wt: improved OS, *P* = .042†
KRAS & NRAS[50]	Panitumumab	1	Genomic (mutations)	Retrospective	Tumor tissue	Sequencing / HPLC	FFPE	1,060	RECIST	RAS wt: improved PFS in panitumumab group, *P* = .004†
										RAS wt: improved OS in panitumumab group, *P* = .009†
BRAF V600E[57]	Cetuximab / panitumumab	≥ 1	Genomic (mutations)	Meta-analysis	—	Sequencing	—	7 studies (3,168)	mWHO	No correlation: PFS, *P* = .07
									RECIST	No correlation: OS, *P* = .43
BRAF V600E[58]	Cetuximab / panitumumab	≥ 1	Genomic (mutations)	Meta-analysis	—	Sequencing	—	9 studies (463)	mWHO	No correlation: ORR, *P* = .25
									RECIST	No correlation: PFS, *P* = .33
										No correlation: OS, *P* = .63
PTL[59]	Cetuximab	1	Clinical	Retrospective	—	—	—	CRYSTAL (364)	mWHO	*RAS wt left-sided:*
										Improved ORR, *P* < .001†
										Improved PFS, *P* < .001†
										Improved OS, *P* = .002†

(Continued on following page)

TABLE 3. Predictive Biomarkers for Response to Anti-EGFR Drugs (Continued)

Biomarker	Anti-EGFR	Line of Therapy	Biomarker Type	Study Design	Specimen	Analytic Method	Sample Type	Discovery Cohort (n)*	Response Criteria	Results†
PTL[59]	Cetuximab	1	Clinical	Retrospective	—	—	—	FIRE-3 (394)	RECIST	*RAS wt left-sided:*
										No correlation: ORR, $P = .23$
										No correlation: PFS, $P < .38$
										Improved OS, $P = .002$†
PTL[60]	Cetuximab / panitumumab	1	Clinical	Meta-analysis	—	—	—	CRYSTAL (364)	mWHO	No correlation: ORR, $P = .2$
								PRIME (416)	RECIST	No correlation: PFS, $P = .3$
										No correlation: OS, $P = .10$
PTL[60]	Cetuximab / panitumumab	1	Clinical	Meta-analysis	—	—	—		RECIST	*RAS wt left-sided:*
								FIRE-3 (394)		Improved ORR, $P = .002$†
								CALGB/SWOG 80405 (474)		No correlation: PFS, $P = .084$
								PEAK (143)		Improved OS, $P = .0003$†

Abbreviations: PTL, primary tumor location; PCR, polymerase chain reaction; HPLC, high-performance liquid chromatography; FFPE, formalin-fixed paraffin-embedded; WHO, World Health Organization; mWHO, modified WHO; RECIST, response evaluation criteria in solid tumors; R, responders; NR, non-responders; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.

*No studies included a validation cohort.

†Statistically significant.

Siravegna et al[74] in 2015. Since then, multiple studies have reported distinct genetic alterations in ctDNA from patients with primary disease or acquired resistance to anti-EGFR–based therapies in genes such as *KRAS*, *NRAS*, *MET*, *ERBB2*, *FLT3*, *EGFR*, and *MAP2K1* by droplet digital polymerase chain reaction, BEAMing, and next-generation sequencing methodologies.[74,75] Using a massively parallel sequencing-based assay in a prospective cohort of 53 patients with mCRC, it was shown that early changes in ctDNA during first-line standard chemotherapy can also predict subsequent radiologic response.[76] Similarly, in 2017, a study demonstrated a significant correlation between the decrease in *RAS* mutant clones in blood after 8 weeks of therapy and improved PFS and OS in a cohort of patients treated with regorafenib.[40] Intriguingly, clonal evolution is a dynamic process, yet the emergence of drug-resistant clones in circulation increases during treatment, whereas drug withdrawal results in a decrease of such clones. The understanding of this fact has paved a path for novel treatment strategies that are already under evaluation as part of the RASINTRO (*RAS* Mutations in ctDNA and Anti-EGFR Reintroduction in mCRC) study (ClinicalTrials.gov identifier: NCT03259009) and the CHRONOS (Rechallenge With Panitumumab Driven by *RAS* Dynamic of Resistance) trial (ClinicalTrials.gov identifier: NCT03227926), which are evaluating the predictive impact of ctDNA *RAS* mutations on the efficacy of anti-EGFR monotherapy rechallenge in patients with *RAS* wild-type mCRC whose disease has progressed after anti-EGFR–free chemotherapy. In addition, a five-gene methylation panel for monitoring tumor burden in liquid biopsies using a methyl-BEAMing assay was described recently[77] in 182 patients with mCRC treated with chemotherapy and/or targeted therapy, in which the authors discovered a significant correlation between the dynamics of methylation markers and ORR and PFS.

DISCUSSION

Despite the tremendous body of effort devoted to the identification of predictive biomarkers for various treatments used in patients with mCRC, thus far only two of such markers have been translated into routine clinical practice. The first one, the mutations in the *RAS* gene, serves as a negative predictive biomarker that is present in approximately 55% of patients with mCRC[78] and correlates with the lack of efficacy of anti-EGFR antibody treatments. The second marker is the tumor MSI status, which has emerged as a predictive marker for anti–PD-1 drugs. The exciting result of the association between MSI-H and response to nivolumab in the first-in-human clinical trial (ClinicalTrials.gov identifier: NCT00441337) led to two subsequent phase II clinical trials, which were instrumental in the approval of anti–PD-1 drugs (pembrolizumab or nivolumab) alone or in combination with ipilimumab (nivolumab plus ipilimumab) as a treatment option for patients with MSI-H or dMMR mCRC.[63-65,79]

Other well-described predictive biomarkers used in the management of several tumor types have shown promising usefulness in selecting patients with mCRC for various targeted therapy-based regimens. Results from two clinical trials in patients with *BRAF V600E*–positive mCRC have highlighted this mutation as a predictive biomarker for BRAF inhibitor–based regimens (Data Supplement).[80,81] Regarding the role of human epidermal growth factor receptor 2 (HER2) amplification or overexpression as a predictive biomarker for anti-HER2–based therapies, the results of two phase II clinical trials evaluating the dual HER2 blockade in a biomarker-selected subset of heavily pretreated patients with mCRC, with either trastuzumab plus lapatinib (HERACLES [HER2 Amplification for Colorectal Cancer Enhanced Stratification] trial) or with pertuzumab and trastuzumab (MyPathway trial), demonstrate an impressive ORR of approximately 30% to 40% (Data Supplement).[82,83]

Nonetheless, the discovery and validation of novel predictive biomarkers that can assist in decision making has been a challenging endeavor, resulting in a long list of failed predictive markers. As highlighted in this article, this task seems even more daunting in terms of conventional chemotherapy and antiangiogenic drugs. In CRC, because the use of single-agent chemotherapeutic regimens has shown limited efficacy, and the majority of current treatment options include various combinations of drugs, biomarker discovery for specific drugs is more complicated, not surprisingly, because of the interactions among different cytotoxic agents.[84] Similar concerns remain regarding developing predictive biomarkers for therapeutic response to bevacizumab, because (1) it is also not used as a single agent in the clinic,[84] (2) its mechanisms of action are poorly understood,[85] and (3) angiogenesis is an intriguingly adaptive process that involves numerous factors.[86] Presumably, the inherent complexity of angiogenesis has been a substantial hurdle in the attempts to develop response-predictive biomarkers for other multitargeted antiangiogenic drugs such as aflibercept or regorafenib. Additional insights into the tumor microenvironment, including the role of tumor-associated stromal cells, could possibly shed light on this tortuous process in the future. The gap between the discovery phase and subsequent biomarker development is evident, highlighting the necessity for the implementation of robust worldwide platforms to move predictive biomarker validation algorithms forward.

Another important question worthy of discussion in any biomarker discovery effort is the origin of tumor tissue samples: primary tumor tissue or metastatic lesions? An interesting example of this important concept is TS expression as a predictive biomarker for FU-based chemotherapy, because its efficacy has been discordant depending on the tumor tissue origin.[4,87] This concept is highly congruent with tumor heterogeneity, which is

a possible source of discrepancy even when the molecular marker is analyzed in a different region of the same source.[88] Besides their spatial heterogeneity, tumors are dynamic entities that continue to evolve over time, especially if they are under selective pressure.[89] For this reason, the time from sample acquisition to biomarker analysis is of clinical relevance; however, this is an issue that is overlooked in most studies. Because only approximately 20% of patients with CRC present with metastatic disease at the time of diagnosis, it is often the practice or only option available to analyze archival tissues from the primary tumor to identify biomarkers, which is not always optimal.[90]

Patient selection is gaining importance, which is evidenced by the recent initiative, the US National Cancer Institute's Exceptional Responder Program.[91] Consideration of extreme phenotypes such as long-term responders and extremely early progressors for biomarker discovery can facilitate successful identification of molecular alterations that better correlate with clinical phenotypes. For instance, in the majority of studies presented in this article, there was no consideration of PFS as a selection criterion, and many studies included in the nonresponders patients with stable disease. In general, improved ORR and longer PFS are superior indicators of the true efficacy of any drug intervention, whereas inclusion of gain in OS as a selection feature may inadvertently introduce bias. In addition, new biomarker-driven study designs such as basket or umbrella trials, which assign a treatment according to tumor molecular characteristics, not only are going to improve clinical drug development, but also will facilitate improved biomarker validation. Besides the examples already described previously in the text, new drugs that target tyrosine kinase fusions in genes such as *NRTK1/2/3*, *RET*, *ALK*, and *ROS1* are emerging with promising preliminary results in phase I and II clinical trials that include patients with CRC (Data Supplement).[92-97]

Although analysis of clinical specimens with robust follow-up data from retrospective series or randomized trials are of tremendous value, subsequent prospective clinical cohorts using longitudinally collected specimens are much needed to establish clinically translatable predictive biomarkers. In addition, although many surgical specimens are of suitable quality, needle biopsy–derived metastatic lesions often yield lower amounts of DNA and RNA than that required for robust sequencing experiments[98,99]; having access to liquid biopsy–based predictive markers would be transformative in overcoming this limitation in patients with mCRC. Furthermore, liquid biopsy biomarkers will improve patient compliance and eliminate the concerns surrounding intratumor heterogeneity associated with tumor or biopsy specimens and may also help in disease monitoring as well as in predicting secondary resistance.

The international community has to consolidate initiatives to improve biomarker development studies and, more importantly, undertake conscious efforts to validate the results gathered from retrospective studies in prospective randomized multicenter cohorts. Such efforts will guarantee improved success and will decrease the economic burden by allowing precision treatment of patients with cancer. Last, the implementation of novel high-throughput molecular analytic techniques and the integration of multiomic approaches with clinical and epidemiologic data using machine-learning algorithms will definitely hasten biomarker development in the coming years.[100]

Despite many attempts over the past decades, there remain only two well-established predictive biomarkers, mutations in the *RAS* gene and MSI status, that currently guide treatment decisions in patients with mCRC. Although past efforts in this context may not have been as rewarding, we currently are at a frontier where the future looks promising. It is just a matter of time until we have access to robust predictive biomarkers for response to cancer therapeutics, as we usher in the new era of precision oncology.

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APPENDIX

("Colorectal Neoplasms"[Mesh] OR "Colorectal Cancer"[tiab] OR "Colorectal Carcinoma"[tiab] OR "Colorectal Neoplasm"[tiab] OR "Colon Cancer"[tiab] OR "Colon Carcinoma"[tiab] OR "Colon Neoplasm"[tiab] OR "Rectal Cancer"[tiab] OR "Rectal Carcinoma"[tiab] OR "Rectal Neoplasm"[tiab]) AND ("Biomarkers"[Mesh] OR "Biomarkers"[tiab] OR "Biomarker"[tiab] OR "marker"[tiab]) AND ("predictive"[tiab] OR "prediction"[tiab] OR "predicting"[tiab] OR "predictor"[tiab] OR "predict"[tiab]) AND ("Fluorouracil"[Mesh] OR "Fluorouracil"[tiab] OR "5FU"[tiab] OR "5-FU"[tiab] OR "oxaliplatin"[Supplementary Concept] OR "oxaliplatin"[tiab] OR "Folfox protocol"[Supplementary Concept] OR "Folfox"[tiab] OR "XELOX"[Supplementary Concept] OR "XELOX"[tiab] OR "CAPOX"[tiab] OR "CAPEOX"[tiab] OR "IFL protocol"[Supplementary Concept] OR "IFL"[tiab] OR "FOLFIRI"[tiab] OR "irinotecan"[tiab] OR "Cetuximab"[Mesh] OR "panitumumab"[Supplementary Concept] OR "Cetuximab"[tiab] OR "panitumumab"[tiab] OR "Anti-epidermal growth factor receptor"[tiab] OR "anti-EGFR"[tiab] OR "Bevacizumab"[Mesh] OR "Bevacizumab"[tiab] OR "anti–vascular endothelial growth factor"[tiab] OR "anti-VEGF"[tiab] OR "antiVEGF"[tiab] OR "anti VEGF"[tiab] OR "aflibercept"[Supplementary Concept] OR "aflibercept"[tiab] OR "antiplacental growth factor"[tiab] OR "anti-placental growth factor"[tiab] OR "anti placental growth factor"[tiab] OR "anti-PIGF"[tiab] OR "antiPIGF"[tiab] OR "anti PIGF"[tiab] OR "Ramucirumab"[Supplementary Concept] OR "Ramucirumab"[tiab] OR "anti-vascular endothelial growth factor receptor-2"[tiab] OR "anti-vascular endothelial growth factor receptor 2"[tiab] OR "antivascular endothelial growth factor receptor-2"[tiab] OR "antivascular endothelial growth factor receptor 2"[tiab] OR "anti-VEGF-R2"[tiab] OR "anti-VEGFR2"[tiab] OR "antiVEGF-R2"[tiab] OR "anti VEGF-R2"[tiab] OR "antiVEGFR2"[tiab] OR "anti VEGFR2"[tiab] OR "regorafenib"[Supplementary Concept] OR "regorafenib"[tiab] OR "Nivolumab"[Supplementary Concept] OR "nivolumab"[tiab] OR "Pembrolizumab"[Supplementary Concept] OR "pembrolizumab"[tiab] OR "anti-programmed death-1"[tiab] OR "anti-programmed cell death protein-1"[tiab] OR "anti-PD-1"[tiab] OR "anti-PD1"[tiab] OR "antiPD-1"[tiab] OR "anti PD-1"[tiab] OR "anti PD1"[tiab] OR "antiPD1"[tiab] OR "TAS-102"[tiab] OR "TAS102"[tiab] OR "trifluridine/tipiracil"[tiab] OR "Response Evaluation Criteria in Solid Tumors"[Mesh] OR "Response Evaluation Criteria in Solid Tumors"[tiab] OR "RECIST"[tiab]) AND ("Response"[tiab] OR "Respond"[tiab] OR "Responder"[tiab] OR "non-Responder"[tiab] OR "non Responder"[tiab]) NOT ("animals"[mesh] NOT "humans"[mesh])

FIG A1. PubMed search query.