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REVIEW ARTICLE

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Biomarkers in colorectal liver metastases: Rising complexity and unknown clinical significance?

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Abstract

Surgical resection of the liver is the standard treatment for colorectal liver metastases, but 70% of patients still experience recurrence, resulting in limited survival. Molecular biomarkers promise guidance within the selection process of individualized treatment and provide better prognostic forecasting of recurrence and response to treatment. Presently, most investigated biomarkers include mutations of *KRAS*, *BRAF*, *TP53*, *PIK3CA*, *APC*, expression of *Ki-67*, and microsatellite instability. As some colorectal cancer tumors exhibit more than one molecular target, in line with a rising number of potential biomarkers, the complexity of their clinical implementation is rising steadily. Therefore, it is important to approach new insights into molecular biomarkers with explicit caution to their clinical applicability and significance, as there are contradictory results arising from multiple available studies and meta-analyses. This review helps to shed light on the complexity of promising biomarkers in both the prognosis and diagnosis of colorectal liver metastases.

KEYWORDS

biomarker, BRAF, colorectal liver metastases, diagnostic, heterogeneity, KRAS, liver resection, mutations, prognostic, RAS

1 | INTRODUCTION

Colorectal cancer (CRC) has 1.8 million cases globally and is the third most common cancer and ranks second by mortality. The incidence is rising due to socioeconomic developments, exposome changes, and the rise in age.[1] In all, 65% of CRC patients will develop metastases and 40% of those occur in the liver. Currently, the majority of CRC patients are assigned to curative surgery followed by adjuvant chemotherapy, which is predominantly determined by clinicopathologic features like primary tumor stage, carcinoembryonic antigen (CEA) level, nodal status, number and size of liver metastases, resection margin status, and the interval between primary tumor diagnosis and liver metastasis.[2,3] Notably, centers offering multidisciplinary treatment approaches including pathologists, radiologists, oncologists, and colorectal as well as liver surgeons show better survival rates than general hospitals or nonspecialized centers.[4] Over 50% of CRC patients will develop colorectal liver metastasis (CLM) and complete surgical removal still offers the best chance for long-term survival.[5] Nonetheless, one-third of CLM patients still succumb because of recurrent disease in the liver, which affects two-thirds of patients after resection.[6,7] Even modern multidisciplinary approaches that entail, e.g., two-stage hepatectomies after portal vein embolization, radiation, multiple ablation techniques for CLM (radiofrequency and microwave ablation), and expanding surgical techniques did not change this situation significantly until now. Currently, perioperative systematic therapy is suggested; however, a large randomized controlled trial by Nordlinger et al involving 364 patients showed no improvement in 5-year overall survival (OS) (51% vs 48%; $P = .34$) in

patients treated with hepatic resection and perioperative therapy with FOLFOX4.[8]

The molecular factors contributing to disease recurrence are still unknown, while histological factors such as tumor grade and differentiation or lymphovascular invasion are the only help in assessing the risk.[6] The molecular events leading to tumor metastasis is immensely complex, and thus a poorly understood process. Mutations at the initial site of colorectal carcinogenesis are critical events in the metastatic process. The events leading to cancer cell invasion, adaptation, and colonization of the hepatic parenchyma, called the colorectal cancer invasion-metastasis cascade, involve various molecular pathways, with inter- and intracellular interactions with potential clinical interest.[9]

From a surgical point of view, there are essentially three predominant clinical scenarios in which validated biomarkers could provide evidence-based guidance in the future:

1. Primarily resectable CLM

For primary resectable CLM, perioperative, individualized therapy must eliminate the micrometastatic disease to prevent recurrent CLM or extrahepatic disease recurrence.

1. Primarily unresectable CLM

Here the primary aim of perioperative therapy is to convert the extent and localization of CLM to a resectable stage of disease. Risk prediction of recurrence and prognosis is of special importance here, since the operative risk must be weighed against expected survival.

1. Synchronous CLM with the primary CRC in situ

The timing and extent of surgical resection is an ongoing discussion in this situation. The question of whether to resect the primary tumor or CLM first is still unanswered.

Hepatic resection is both the major aim and challenge in the treatment process of CLM. The challenge itself lies in the need for a stratified selection of patients feasible for surgery, which is still mainly based on personal decision-making by the surgeons. This clinical complexity is possibly also expressed in the wide ranges of the 5-year OS after CLM (25%–58%).[10,11] Due to the genetically heterogeneous nature of colorectal cancer with CLM, the diagnosis, prognosis, and selection for treatment are, therefore, challenging for surgeons.[6] An individualized therapy based on molecular profiling of the genetic landscape is highly required to improve survival rates and avoid an unnecessary burden on the patient as well as the healthcare system. Unfortunately, to date no biomarker has been translated into clinical practice to guide either exclusion of patients from surgery or for the timing of surgery.

The molecular biomarker's potential to evaluate tumor biology by noninvasive methods are promising (see Figure 1), but the definite effectiveness in clinical settings remains unclear. This review aims to provide an overview of the most emergent biomarkers in the multidisciplinary treatment approach of CLM to illustrate evidence-based treatment opportunities after molecular stratification, focusing especially on the clinical application.

2 | METHODS

This review summarizes and assesses research findings in the English written literature published between 1999 and 2020, with a special focus on the most up-to-date research findings using the PubMed

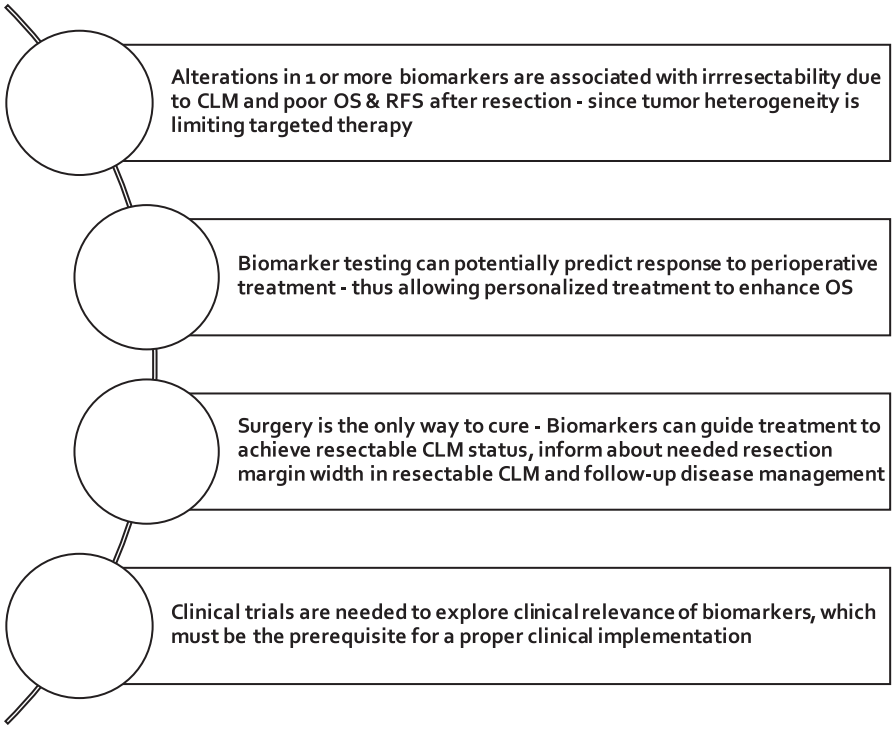

FIGURE 1 Highlights: Biomarkers and their clinical importance. CLM, colorectal liver metastases; OS, overall survival; RFS, recurrence-free survival

database. Keywords and cross-references of prior published literature including reviews and meta-analyses were used. Search strings included colorectal, liver, metastasis, biomarker, RAF, KRAS, BRAF, resection in various AND combinations.

2.1 | Sidedness of the primary tumor

When making a treatment decision for the side of the primary tumor in the colon is a factor to consider since tumors show different molecular features. Overall, patients with left-sided tumors have better survival with fewer metastases and are more often suited for curative resection.[12] Taniguchi et al[13] showed that right-sided tumors are more likely to expose mutations of *KRAS* and *BRAF*, which correlate with unresectable liver metastases status, resistance to anti-EGFR (epidermal growth factor receptor) antibody treatment, and negative prognostic outcome after liver resection. *BRAF* mutations occur in 32.3% of cases on the right-sided colon. Additionally, right-sided tumors with wildtype *RAS* show a high prevalence (17.2%) of *PIK3CA* mutations. Thus, testing for those markers in right-sided tumors can be recommended.[14]

2.2 | Heterogeneity of primary tumor and metastases

Even though initially carrying identical mutations, further mutations result in genetic heterogeneity between the primary tumor and metastases (intertumor heterogeneity). Thus, primary tumor and distant metastases must be considered genetically heterogeneous.[15]

A high degree of primary CRC heterogeneity (intratumor heterogeneity), including mutations in *APC*, *TP53*, and *KRAS* is associated with liver metastasis and poor response to therapy and survival. Tumor heterogeneity helps metastatic CRC to develop collectively through the parallel spread of multiple clonal subpopulations via vascular invasion.[16]

Moreover, a high level of intermetastatic genomic heterogeneity in the same patient (intrapatient heterogeneity) might cause worse outcome factors after hepatic resection, including chemoresistance.[17]

2.3 | Somatic gene alterations in CLM

Alterations in genes like *KRAS*, *P53*, *BRAF*, and *PIK3CA* frequently influence tumor behavior in CLM. Here we look at the latest research performed around these genes in CLM, and lastly into the crosstalk of the genes that are believed to explain the inconclusive and contradicting study results.

2.3.1 | RAS Proto-oncogenes

Rat sarcoma viral oncogene homologs (RAS) are oncogenes assessed in CLM typically in codons 12, 13, 61, and 146. Oncogenes are mutated or upregulated forms of proto-oncogenes, typically causing gain-of-function and uncontrolled cell proliferation. Mutations in *KRAS*, NRAS, HRAS, proto-oncogenes of the *RAS* family lead to an active MAPK pathway, which results in resistance to preoperative systemic treatment with the EGFR inhibitor (cetuximab).[18,19] Thus, *RAS* status is a validated predictor of response to chemotherapy targeting EGFR.[19-21] *RAS* mutations, from either primary tumor or liver metastases, are the most researched and known negative prognostic biomarkers for patients with CLM resection associated with poor OS.[22] Brudvik et al[18] showed in their systematic review a 3-year OS of 52% in *RAS* mutated patients, while wildtype patients reached 81%. Prospective studies confirmed the relationship between *RAS* mutations and poor outcomes after resection of CLM.[18] For that reason, testing of *RAS* mutational status in combination with other clinical-pathological factors can be advised to estimate tumor response to chemotherapy. Still, conflicting results in the literature mirror the complexity of the topic. Kawaguchi et al[23] found that *RAS* mutation status alone is not adequate for the prognosis after resection of the liver, but multiple somatic mutations in the genes *RAS*, *TP53*, and *SMAD4* were associated with worse prognosis than a single mutation after resection of CLM and patients with unresectable colorectal metastases.

2.3.2 | KRAS

Kirsten rat sarcoma viral oncogene homolog (KRAS) is an oncogene belonging to the GTPase *RAS* superfamily and triggers pathways involved in cell proliferation and differentiation as a growth signal transducer downstream of the EGFR. Thus, when over-active, caused by alteration, *KRAS* is involved in neoplastic transformation as an early event in colorectal carcinogenesis. *KRAS* is found to be mutated in 30%–45% of CRC, 25%–52% of CLM tumors[20] and has a high concordance between primary tumor and liver metastases.[24] In patients undergoing liver resection for CLM, *KRAS* has been shown to be a significant predictor of OS and recurrence-free survival (RFS).[5,21,25-28] For instance, in one study, using a large national multicenter web-based database including 622 patients, *KRAS* mutation was an independent predictor of death or recurrence in CLM, expressed as RFS, which was 22% at 5 years for mutant *KRAS (mt-KRAS)* and 33% at 5 years for wildtype *KRAS (wt-KRAS)* (P =.0053; hazard ratio [HR]: 1.42). Both *wt-KRAS* and *mt-KRAS* patients received pre- and postoperative systemic treatment.[29]

To achieve a microscopically margin-negative resection (RO) in multiple bilobar metastases surgery, meaning no tumor tissue remains in the resection margin, a large proportion of normal parenchyma needs to be resected. This leads to an increased risk of liver failure after surgery and potential morbidity and mortality.[4] Here, the resection margin status is of relevance in *wt-KRAS* but shows no prognostic relevance in *mt-KRAS*.[30] There is a positive correlation between *mt-KRAS* and high incidence of micrometastases, resulting in insufficient safety resection margins. Moreover, patients who received preoperative chemotherapy are associated with a lower incidence of

micrometastases and were more likely to receive surgical resection after tumor shrinkage. Thus, a wider resection margin might be recommended in patients with *mt-KRAS* to achieve RO resection.[31]

Brunsell et al[22] showed that KRAS mutations in multiple resected CLMs were of individual prognostic significance of OS in addition to being a predictive marker for the effect of anti-EGFR therapy in CLM independent of the systemic treatment.

In this study, most patients with primarily resectable CLM received adjuvant, neoadjuvant, and oxaliplatin-based chemotherapy. Other patients received conversion treatment including targeted agents to achieve resectable CLM status. In patients with primarily resectable liver metastases, no clinical benefit of anti-EGFR antibodies has been recognized.[22]

2.3.3 | BRAF

The v-raf murine sarcoma b-viral oncogene (BRAF) gene is another member of the *RAS* family and plays, as a protein kinase, a crucial role in the mitogen-activated protein kinase APK/ERK signaling pathway. *BRAF* activation is associated with cell differentiation, migration, angiogenesis, and proliferation. *BRAF* gene products act downstream of *KRAS* in the MAPK signaling pathway. A valine-to-glutamic acid amino acid in codon 600 (V600E) substitutions leads to the abnormal activation of the MEK–ERK pathway. *BRAF V600E* is found to be the most important alteration—making up 90% of all *BRAF* mutations and an indicator of aggressive disease.[32]

BRAF mutation (mt-BRAF) is found in 15%–35% of patients with resectable CLM and correlates with worse OS and RFS after liver resection.[20] Because only a few *mt-BRAF* patients are eligible for liver surgery, clinical studies with resectable CLM are sparsely available, as these patients are usually treated by palliative chemotherapy. The newest evidence states that BRAF mutations do not increase the risk of recurrence after resection of CLM resected patients; however, if recurrence occurs *mt-BRAF* is associated with poor survival.[33] However, *mt-BRAF* has also been shown to be a biomarker in determining the response to anti-EGFR antibodies.[34,35]

BRAF and *PIK3CA* mutation is found to be more prevalent in right-sided CRC and is shown in the resistance to anti-EGFR therapy.[13,32]

2.3.4 | Ki-67

Ki-67, a nuclear non-histone protein, is found in all active phases of the cell cycle and drives cellular proliferation. Increased expression over 50% is linked to a negative prognosis, shown in a lower median survival after hepatic resection. High *Ki-67* expression has been reported in 19.5%–62% of patients with CLM. A study on liver metastases samples of 124 patients with resected CLM revealed that high *Ki-67* expression may be an even stronger predictor of prognosis than KRAS; interestingly, patients with high *Ki-67* expression were more likely to present with synchronous CLM, high tumor burden, and preoperative CEA >200 ng/mL and less likely to undergo curative-intent resection. On the contrary, a retrospective analysis with 98 liver resection patients reported conflicting results, where *Ki-67* overexpression was a positive prognostic factor of survival.[36] The explanation is believed to be found in the study design: the later study did include subjects that were chemotherapy-naive, had multiple metastases, and a short time interval to metastasis, while the other did not differentiate.[34]

2.4 | Multiple alterations

Variable and conflicting results in available studies are most likely to be based on the above-mentioned complexity and under-researched interactions of multiple genes, their alteration status, and the different individual cancer-related pathways. However, mutations occurring in multiple genes are likely to be associated with negative OS and RFS in most studies.

KRAS mutations together with mutations in *NRAS (Neuroblastoma RAS viral oncogene homolog)* (exons 2, 3, and 4), another proto-oncogene, are predicting the limited treatment efficacy of anti-EGFR monoclonal antibodies (mAb) cetuximab and panitumumab due to resistance. The clinical practice guidelines in Europe and the USA involve indications for *RAS* testing *(KRAS and NRAS mutations)* before the use of anti-EGFR agents.[37,38] Co-alterations in *RAS* and *BRAF* are observed rarely in colorectal liver metastases, with only 0.05%. A systematic review covering 11 publications found that coaltered *RAS* and *BRAF* show different genetic signatures, suggested in molecular profiling; however, no study has proven the role in metastatic disease so far.[39]

TP53, together with *RAS* alteration, is also associated with worse survival. In one study by Kawaguchi et al[23], including 485 patients, OS and RFS after CLM resection were worse in patients with co-alteration in *RAS* and *TP53* than in patients with one alteration of the two genes and patients with no alteration. Consequently, the authors suggested that even after the 2 years of recurrence-free CLM resection, patients with co-alteration should receive a different, RFS-focused disease management, considering repeat resection and/or chemotherapy, determining surveillance frequency and intensity, and scheduling clinical surveillance more frequently and intensely. A meta-analysis of seven trials with 1403 patients concluded that both *KRAS* and *BRAF* were negative prognostic factors in patients with hepatic resection of CLM compared to wildtype. Together with other clinical-pathological factors, assessment of biomarkers could potentially help to predict recurrence and survival after surgical resection. Yet the particular studies had different selection criteria and samples from either primary tumor or liver metastases, and thus proper interpretation is limited.[27]

2.4.1 | APC & PIK3CA

Adenomatous polyposis coli (APC) is a tumor suppressor gene inducing apoptosis. Loss of function mutation is stimulating the formation of

tumors and appears in 47.4% and 48.7% of CLM. However, regarding the prognosis for CLM liver resection, *APC* together with *PIK3CA* mutations have been shown to result in lower OS and RFS compared to single mutations only. It is important to note here that more studies are needed to confirm this statement.[34]

Phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) regulates cell proliferation. Studies found a mutation rate of 13.4%–20.9% in CLM patients, 80% of mutations are in exons 9 and 20, whose concomitant existence has been reported to have been linked to poor prognosis. Furthermore, a poor prognostic outcome has been reported for *PIK3CA* mutation in combination with *wt-Kras* status.[40] Taken together, those biomarkers are currently not suitable for clinical use, since more research is badly needed.

2.4.2 | Microsatellite instability

Deficient DNA mismatch repair (dMMR) leads to microsatellite instability (MSI) and is associated with less metastatic potential and thus a better prognosis of survival. MSI-high tumors occur in only 5% of metastatic colorectal cancer, but correspond in 34.4% of cases with a simultaneous presence of BRAF V600E mutations. The MSI-high and *BRAF V600E* mutant combination shows a better 5-year survival (73%) compared to the microsatellite stable phenotype combined with *BRAF V600E* wildtype (65%). MSI-high with *BRAF V600E* wildtype shows the best prognosis of a 75% 5-year survival. The data suggest therefore a combined testing for MSI & *BRAF*.[41] The use of immunotherapy for patients with MSI-high metastatic colorectal cancer as well as preoperative therapy before CLM resection has been studied in clinical trials.[32] The National Cancer Institute recommended a panel of five microsatellite loci for evaluating MSI status in either high-frequency MSI (MSI-H) and low-frequency MSI (MSIL). MSI-H CRC is mostly located in the right-sided colon and defined by mucinous features, poor differentiation, and lymphocytic invasion. MSI-H shows less risk of distant recurrence, while MSI-L patients show a lower OS than patients with MSI-H. Immune checkpoint inhibitors (ICI), also known as checkpoint immunotherapy, proved to be effective in up to 50% of the 13%–16% CLM patients who exhibit dMMR or MSI-H. Therefore, testing for microsatellite instability can predict prognosis and response to ICI therapy.[42]

3 | DISCUSSION

In this review we aimed to focus on the challenge of resectable and unresectable CLM disease management with a focus on the complexity and potential clinical impact of molecular biomarkers. Prediction of the risk of recurrence after resection of CLM is of special importance for informed decision making intended for the right individualized treatment plan that enables greater survival chances, less risk, and cost-effectiveness. Moreover, emerging noninvasive molecular

TABLE 1 Biomarkers clinical impact for diagnostic, prognostic, and therapy in patients with CLM

Biomarker	Prevalence (CLM)	Diagnostic	Prognostic/prediction	Therapeutic
RAS	30% left-sided,48.2% right-sided	Testing RAS + other clinico-pathological factors	Poor OS, 52% vs 81% wt-RAS after resection; earlier metastatic disease; EGFR antibodies response prediction; worse preoperative chemotherapy response	Wider cross-resection margins of 15 mm suggested and a wider ablation margin
BRAF	5%–11%	Testing to predict anti EGFR response & prognosis	Poor OS + RFS after resection; no benefit of anti EGFR antibodies	Wider safety margins needed to achieve Ro resection; resection asap after first-line therapy response
KRAS	29.5% left-sided; 46.9% right- sided	Testing to predict anti-EGFR response & prognosis	Poor OS + RFS after resection; resistance to anti-EGFR antibodies	Multidisciplinary treatment after curative resection of CLM
Ki-67	19.5%–62%	Less often undergoing resection due to poor clinic-pathological factors	expression over 50% associated with lower median survival after hepatic resection	Multidisciplinary treatment before and after curative resection of CLM
APC & PIK3CA double mutation	42%–73% & 6.7%–28%	Testing	predicts poor response to preoperative chemotherapy and poor survival in patients with CLM	Personalized treatment to achieve resectable CLM

biomarkers could help to predict response to various chemotherapeutics and anti-EGFR antibodies, detect, predict, or decrease recurrence after curative resection earlier, and minimize treatment toxicity. Further benefits include enabling effective complements to first-line standard therapies and individualized second-line therapies and give a better prognosis of survival (see Table 1).

Further studies and sound clinical trials are needed to develop a cost-efficient and clinically effective molecular biomarker screening plan for the fast application of research data into clinical practice for patients with CLM. The complexity of the genetic mutations and the resulting variability of the tumor behavior make this a challenging task. Most identified biomarkers, apart from *KRAS* and *BRAF*, did not prove an effectiveness in the majority of available studies because of the molecular heterogeneity of tumors and metastases, and underpowered study design. Likewise, publication bias is an important factor to consider before making a conclusive interpretation. Existing, partly overlapping meta-analyses also exhibit a significant heterogeneity of included and excluded studies—urging for caution when interpreting the results.

The current body of evidence clearly shows an urgent need for prospective trials as well as prognostic and diagnostic studies to accomplish reproducible results of clinically relevant gene signatures in the treatment of patients with CLM. This claim is underpinned by the fact that even the role of the best-explored entities such as *KRAS* and *BRAF* mutations remains debatable in the clinical real-world situation. In other words, there is still not enough solid evidence that *KRAS* or *BRAF* mutated patients are less likely to obtain a benefit from standard chemotherapeutic strategies. While most data from nonrandomized, retrospective studies, report *KRAS* status as a potential application as prognosis biomarkers in CRC management,[43-45] other studies do not.[21,46] Nevertheless, *KRAS* status has a strong potential for clinical implementation as a prognostic biomarker other than prediction to an anti-EGFR response. A large meta-analysis by Brudvik et al[5] combined data of 14 distinct studies researching *KRAS* status outcome after resection of CLM. This analysis showed an increased risk of recurrence (hazard ratio [HR] 1⁄4 1.89, 95% confidence interval [CI] 1.54e2.32) and worse OS (HR 1⁄4 2.24, 95% CI 1.76e2.85) in *mt-KRAS* vs *wt-KRAS* after resection.[5]

While surgery of the liver is considered today still the only potentially curative treatment for CLM, the risks of an extensive liver resection should be outweighed by the benefits of this surgery. Biomarkers could help to make those decisions easier and more effective by tailoring the therapy to molecular profiling and to avoid risky treatment without a real survival benefit. Thus, especially a metastatic liver disease bears the potential to benefit from these novel biomarkers in diagnosis, prognosis, and stratifying treatment approaches, although there is more knowledge needed from clinical research for validating specific biomarkers. Last, biomarkers were studied in CLM patients, as well as the comparable high frequency in liver metastases, and thus comparable sufficient subjects could be enrolled in the studies. Hence, the implication of biomarkers as prognostic and diagnostic tools remains promising but needs proper further investigation.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest for this article.

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