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ORIGINAL ARTICLE

Diagnostic Accuracy of BRAF Immunohistochemistry in Colorectal Cancer: a Meta-Analysis and Diagnostic Test Accuracy Review

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Abstract The aim of this study was to evaluate the concordance between the *BRAF*^{V600E} mutation test and immunohistochemistry (IHC) and to evaluate the diagnostic accuracy of BRAF IHC for colorectal cancer (CRC) through a systematic review, meta-analysis, and diagnostic test accuracy review. The current study included 1021 CRCs from eight eligible studies. The concordance rates were investigated between BRAF IHC and the mutation test. In addition, diagnostic test accuracy review was conducted and calculated using the value of area under curve (AUC) on the summary receiver operating characteristic (SROC) curve. The positive rate of BRAF IHC was 30.5 % (range; 13.2–66.2 %), and the *BRAF* mutation was found in 30.2 % (range; 11.7–66.2 %). The overall concordance rate between BRAF IHC and the mutation test was 0.944 (95 % confidence interval (CI) 0.873–0.977). In the BRAF IHC-positive and -negative groups, the concordance rates between BRAF IHC and the mutation test were 0.895 (95 % CI 0.800–0.945) and 0.956 (95 % CI 0.878–0.985), respectively. The pooled sensitivity and specificity were 0.94 (95 % CI 0.91–0.96) and 0.96 (95 % CI 0.95–0.98), respectively. The diagnostic odds ratio was 272.86 (95 % CI 46.11–1614.88), and the value of AUC on SROC curve was 0.9846. Taken together, our results suggest that BRAF IHC is strongly concordant with the *BRAF* mutation test and has high diagnostic accuracy in *BRAF* mutation analysis of CRCs. Further cumulative studies on detailed evaluation criteria are needed before application in daily practice.

Keywords *BRAF*^{V600E} immunohistochemistry · Colorectal cancer · Concordance · Diagnostic accuracy · Meta-analysis

Abbreviations

BRAF	B-type Raf kinase
CRC	Colorectal cancer (CRC)
IHC	Immunohistochemistry
SROC	Summary receiver operating characteristic
AUC	Area under the curve
OR	Odds ratio

Introduction

The protein kinase B-type Raf kinase (BRAF) is involved in the RAS/RAF/MEK/ERK signaling pathway and regulates cell growth and propagation in response to extracellular signals in human cancers [1]. Various *BRAF* mutations, such as V600E, V600 K, V600 M, V600R, V600D and V600G, have been reported, and the *BRAF*^{V600E} mutation is the most frequent [2, 3]. *BRAF* mutations are found in various malignant tumors, including colorectal cancer (CRC) [4–11], melanoma [12], papillary thyroid carcinoma [1], and hairy cell leukemia [13]. The prevalence and clinicopathological significance of *BRAF* mutations are variable according to tumor type. *BRAF* mutation is found in 100 % of cases of hairy cell leukemia [13]. However, *BRAF* mutation is identified in up to 60 % and 90 % of cases of malignant melanoma and papillary thyroid carcinoma, respectively [1, 14, 15]. In CRCs, *BRAF* mutation rates have been reported in the range of 5–25 % [16–18]. Detection of *BRAF* mutation is important to differentiate

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sporadic cases from Lynch syndrome. In addition, *BRAF* mutation analysis could be useful for predicting prognosis and response of anti-EGFR therapy in CRC [19].

The *BRAF* mutation is detected through various PCR-based molecular tests. However, these methods are expensive, multi-step, time-consuming, and require DNA extraction. In addition, the impact of inclusion of non-tumoral cells on diagnostic accuracy should be considered. Because immunohistochemistry (IHC) does not require DNA extraction or purification, the effect of this non-tumoral component may be ignored. Moreover, IHC is one of the most popular molecular and morphologic methods in most pathologic laboratories. Recently, BRAF IHC using mouse anti-human $BRAF^{V600E}$ monoclonal antibody (clone VE1) was introduced, and detection of *BRAF* mutation using BRAF IHC has been reported. BRAF IHC in papillary thyroid carcinoma showed high concordant rates with the *BRAF* mutation test and high diagnostic accuracy for *BRAF* mutation [20].

Immunohistochemical staining is easily applicable in daily practice; however, the diagnostic accuracy of BRAF IHC in CRCs has not yet been verified. Therefore, for the application of BRAF IHC in CRCs, the concordance between BRAF IHC and the mutation analysis test and the diagnostic accuracy of BRAF IHC require investigation. The current study performed a meta-analysis and diagnostic test accuracy review to elucidate the diagnostic usefulness of BRAF IHC in CRCs.

Materials and Methods

Published Studies Search and Selection Criteria

Relevant articles were obtained by searching the PubMed and MEDLINE databases up to August 31, 2015. Searching was performed using the following key words: 'colon or colorectal cancer,' 'BRAF,' and 'immunohistochemistry.' The title and abstract of all searched articles were screened for exclusion. Review articles were also screened to find additional eligible studies. The search results were then reviewed according to the following inclusion and exclusion criteria: (1) *BRAF* mutation was investigated in human colorectal tissues, (2) the correlation between BRAF IHC and mutation test was included, (3) case reports or non-original articles were excluded, and (4) all were English language publications.

Data Extraction

Data from all eligible studies were extracted by two authors. The following data were extracted from each of the eligible studies [4–11]: first author name, year of publication, study location, type of specimen, manner of $BRAF^{V600E}$ mutation test, dilution ratio of antibody, antibody clone and manufacturer, and number of patients analyzed. For the meta-analysis, we extracted all data associated with BRAF IHC results (positive and negative) and *BRAF* mutation test results (number of mutation and wild type).

Statistical Analysis

To perform the meta-analysis, data were analyzed by the Comprehensive Meta-Analysis software package (Biostat, Englewood, NJ, USA). We investigated the *BRAF* mutation rate according to immunohistochemical results for the meta-analysis. Concordance rates were measured the rates of coincidence results between BRAF IHC and the mutation test. The weakly positive subgroup defined as an equivocal case with weak intensity, like HER2 IHC score 2+ cases in gastric and breast cancers. To confirm the diagnostic role of weakly positive cases, subgroup analysis was conducted based on the intensity of BRAF IHC. Heterogeneity between studies was checked using the Q and I^2 statistics, and presented using *P*-values. Additionally, a sensitivity analysis was conducted to assess the heterogeneity of eligible studies and the impact of each study on the combined effect. For assessment of publication bias, Begg's funnel plot and Egger's test were performed. The results were considered statistically significant when $P < 0.05$. Moreover, diagnostic test accuracy review was carried out using the Meta-Disc program (version 1.4) (Unit of Clinical Biostatics, the Ramon y Cajal Hospital, Madrid, Spain) [21]. The pooled sensitivity and specificity were gathered sensitivity and specificity from each eligible study, and of which, forest plots were obtained. The summary receiver operating characteristic (SROC) curve was initially constructed by plotting 'sensitivity' and '1-specificity' of each study and the curve fitting was performed through linear regression using the Littenberg and Moses linear model [22]. Because heterogeneity by evaluation criteria was present, the accuracy data were pooled by fitting a SROC curve and measuring the value of the area under the curve (AUC) [21]. An AUC close to 1 will be a perfect test and an AUC close to 0.5 will be considered as poor tests. In addition, the diagnostic odds ratio (OR) was calculated by the Meta-Disc program.

Results

Selection and Characteristics of Studies

In the current systematic review and meta-analysis, 125 reports were identified in the database search. Among the searched reports, 108 were excluded due to lack of or insufficient information on the correlation between BRAF IHC and the mutation test. In addition, nine reports focused on other diseases (3) or were case reports (3), non-original articles (2), or articles in a language other than English (1) and were excluded. Eight eligible studies were ultimately included, and a

meta-analysis was performed for 1146 patients (Fig. 1 and Table 1) [4–11]. Anti-human BRAF^{V600E} monoclonal antibody (clone VE1, Spring Bioscience, Pleasanton, CA, USA) for IHC was used in all eligible studies.

Concordant Analysis between BRAF IHC and the Mutation Test

The overall *BRAF* mutation rate was 30.2 % in 1021 CRC patients. The range of *BRAF* mutation rates was 11.7 %–62.2 %. The overall BRAF IHC-positive rate was 30.5 % (312 of 1021) and ranged from 13.2 % to 62.2 % in eligible studies. The overall concordance rate, including the BRAF IHC-positive and -negative cases, was 0.944 (95 % CI 0.873–0.977). In the IHC-positive and -negative groups, the concordance rate between BRAF IHC and the mutation test was 0.895 (95 % CI 0.800–0.948) and 0.956 (95 % CI 0.878–0.985), respectively (Table 2). To elucidate the cause of the lower concordance of BRAF IHC-positive group compared to the IHC-negative group, subgroup analysis was conducted based on the intensity of BRAF IHC. In the subgroup analysis, the concordance rate was 0.386 (95 % CI 0.159–0.677) in the weakly positive subgroup. Sensitivity analysis showed that eligible studies had no effect on the pooled concordance rates in either the IHC-positive (range; 0.880–0.914) or -negative group (range; 0.934–0.968). In the assessment of publication bias, Egger's test showed no evidence of publication bias, and no definite asymmetry was identified in Begg's funnel plots.

Diagnostic Test Accuracy Review of BRAF IHC

We evaluated the diagnostic accuracy of BRAF IHC in CRCs using a diagnostic test accuracy review. The pooled sensitivity and specificity were 0.94 (95 % CI 0.91–0.96) and 0.96 (95 % CI 0.95–0.98), respectively (Fig. 2). The ranges of sensitivity and specificity were 0.71 to 1.00 and 0.65 to 1.00, respectively. The pooled diagnostic OR was significantly high at 272.86 (95 % CI 46.11–1614.88). The value of AUC on the SROC curve was 0.9846, nearing 1 (Fig. 3).

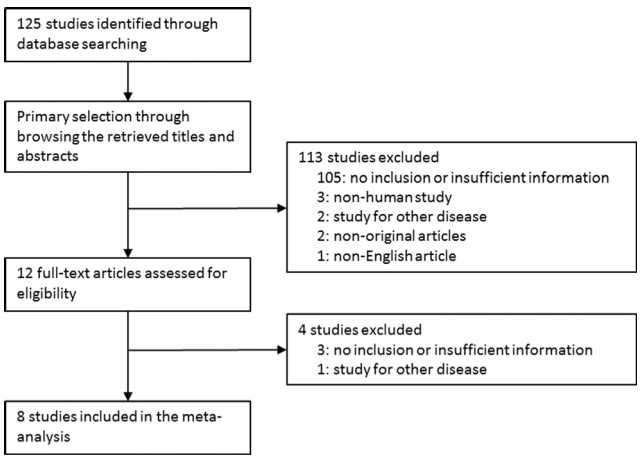

Fig. 1 Flow chart of study search and selection methods

Discussion

Various personalized treatments based on molecular analysis have been applied among CRC patients. The *BRAF* mutation test in CRC is useful for differentiation between Lynch syndrome and sporadic cases and for prediction of response to anti-EGFR therapy [19]. Although the BRAF VE1 antibody has been recently introduced and studied for its usefulness in daily practice, it is not yet fully understood. The current study is the first meta-analysis and diagnostic test accuracy review of published studies of the diagnostic accuracy of BRAF IHC in CRC. There were three major findings in the current study. First, the concordance rate between the *BRAF* mutation test and IHC was high (0.944). Second, the concordance rate of the BRAF IHC weakly positive subgroup was significantly lower than the concordance rate of overall BRAF IHC positive cases, like HER2 equivocal cases of breast and stomach cancers [23, 24]. Third, in the diagnostic test accuracy review, there was no difference in diagnostic accuracy between BRAF IHC and the mutation test.

BRAF mutation rates have been reported in the range of 5–25 % [16–18]. However, in our systematic review and meta-analysis, the overall *BRAF* mutation rate was 30.2 %. This discrepancy may be caused by the composition of patients and differences in detection methods. At present, the gold standard for *BRAF* mutation analysis is PCR-based molecular testing with DNA extraction. These PCR-based *BRAF* mutation tests are more expensive, require multiple steps, and are time-consuming. However, unlike PCR-based tests, IHC is easily and widely used in daily practice in most pathologic laboratories. Recently, the anti-human *BRAF*^{V600E} mutation monoclonal antibody VE1, which directly detects the *BRAF* V600E mutated amino acid sequence from amino acids 596 to 606 (GLATEKSRWSG), was introduced, and studies of BRAF IHC in various cancers have been conducted [7, 12]. Individual studies have been investigated for BRAF IHC VE1 using various types of samples and methods. However, the established methods and evaluation criteria of BRAF IHC have not been fully elucidated. In our previous study, we reported that BRAF IHC using clone VE1 is well concordant with PCR-based *BRAF* mutation tests in papillary thyroid carcinoma [20]. In CRCs, a meta-analysis and diagnostic test accuracy review are needed for evaluation of the usefulness of this new testing method.

In our diagnostic test accuracy review, the value of AUC on SROC was 0.9846, nearing 1. In addition, the diagnostic OR was significantly high, at 272.86 (95 % CI 46.11–1614.88). However, Adackapara CA et al. reported that the sensitivity and specificity of BRAF IHC for *BRAF* mutation were 71 %

Table 1 Main characteristics of the eligible studies

Study	Country	Period of study	Type of sample	N	Criteria	IHC Positive rate	*BRAF* Mutation rate	False IHC positive	False IHC negative	*BRAF* mutation in weak positive
Adackapara et al. 2013 [4]	USA	2006–2012	Whole	34	ND	52.9 %	50.0 %	6/18	5/16	6/12
Affolter et al. 2013 [5]	USA	2008–2012	Mixed[a]	31	ND	45.2 %	45.2 %	0/14	0/17	2/2
Day et al. 2015 [6]	Australia	ND	TMA	477	ND	13.2 %	11.7 %	8/63	1/413	–
Dvorak et al. 2014 [7]	Australia	ND	TMA	279	85 %	31.9 %	30.8 %	5/89	2/190	–
Lasota et al. 2014 [8]	Poland	ND	TMA	55	ND[b]	40.7 %	41.6 %	4/46	5/67	1/12
Nolan et al. 2014 [9]	Canada	1995–2009	TMA	16	80 %	27.7 %	24.1 %	6/23	3/60	3/8
Roth et al. 2015 [10]	USA	2009–2013	Mixed[a]	55	ND	50.9 %	58.2 %	0/28	4/27	–
Sinicrope et al. 2013 [11]	USA	ND	Whole	74	70 %	66.2 %	66.2 %	0/49	0/25	–

N Number of patients, *IHC* immunohistochemistry, *Whole* Whole section, *TMA* Tissue microarray, *ND* No description

[a] Mixed; included biopsied, whole section and tissue microarray specimens

[b] Used 4-tiered system

and 74 %, respectively [4]. Although the impact of different immunohistochemical methods on this discrepancy is not definitive, that study used different methods for antigenicity retrieval and staining compared to other eligible studies. However, in our sensitivity analysis, there was no effect of removal of the Adackapara et al. study [4] on the pooled estimates. The current diagnostic test accuracy review showed higher pooled sensitivity (0.94, 95 % CI 0.91–0.96) and specificity (0.96, 95 % CI 0.95–0.98) for prediction of *BRAF* mutation through BRAF IHC. Thus, the possibility of *BRAF* mutation is significantly higher in BRAF IHC-positive cases than in BRAF IHC-negative cases, and BRAF IHC can replace the PCR-based *BRAF* mutation test.

Basically, in BRAF IHC using the VE1 antibody, cytoplasmic expression, regardless of nuclear expression, is considered to be positive. However, there are no known or detailed criteria for interpretation of BRAF IHC in CRC. In addition, there has been no conclusive information on the discrepancy of BRAF IHC between small biopsied specimens and surgically resected specimens. In small biopsied specimens, the sampling error should consider tumor heterogeneity. When the tissue microarray specimen was a small biopsied specimen, the concordance between BRAF IHC and mutation test was 0.954 (95 % CI 0.895–0.981, data not shown), slightly higher than the overall concordance rate. However, the impact of specimen type could not be determined in the present study due to lack of definitive information on specimen type in most eligible studies. Although the accuracy of *BRAF* mutation analysis can be affected by non-tumoral components due to DNA extraction, there is no effect of non-tumoral components in BRAF IHC staining. In a previous study, nuclear expression of BRAF VE1 was reported and found to be a common feature in CRCs [4]. However, in other eligible studies, no clinicopathological significance of nuclear expression was found. Several technical issues, such as tissue preservation and fixation, may have an effect on BRAF VE1 nuclear staining [7]. Technically, because the BRAF VE1 antibody binds to a specific site, nuclear staining of BRAF VE1 may be considered to be a non-specific finding.

In the current meta-analysis, the discordance rate in BRAF IHC-positive cases was 0.105. The causes for discordance between BRAF IHC and the mutation test are variable, including tumor type, technical issues, and interpretation discrepancy. In Vakiani's report, one discordant case between *BRAF* mutation test and IHC was a signet ring cell type tumor [25], which could be an example of discordance by tumor type. In

Table 2 Concordant analysis between BRAF immunohistochemistry and mutation test

Stratified analysis	Number of studies	Number of patients	Fixed effect [95 % CI]	Heterogeneity [*P*-value]	Random effect [95 % CI]	Egger's test
Overall	8	1021	0.933 [0.913, 0.950]	< 0.001	0.944 [0.873, 0.977]	0.592
IHC positive	8	330	0.873 [0.823, 0.910]	0.004	0.895 [0.800, 0.948]	0.183
Weakly positive only	4	34	0.401 [0.234, 0.595]	0.119	0.386 [0.159, 0.677]	0.954
Weakly positive excluded	8	296	0.913 [0.867, 0.944]	0.153	0.925 [0.863, 0.960]	0.118
IHC negative	8	816	0.930 [0.894, 0.955]	< 0.001	0.956 [0.878, 0.985]	0.135

IHC Immunohistochemistry, *CI* Confidence interval

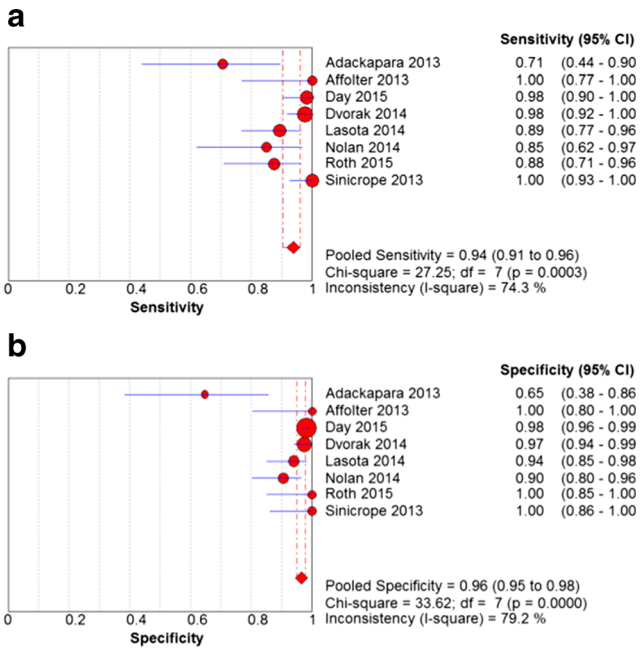

Fig. 2 The sensitivity (**a**) and specificity (**b**) of BRAF immunohistochemistry in colorectal cancer

addition, Dvorak et al. reported that fixation time affected the intensity of the BRAF signal in xenograft [7]. In the current systematic review, some eligible studies used a higher positive criterion of 70–85 % [7, 9, 11]. Because the criterion for BRAF IHC positivity was higher than in other eligible studies, more false negative or equivocal cases may have occurred in that study. In daily practice, consideration of false negative cases may be more important in treatment applications. An effort to decrease the false negative rate is needed. Furthermore, intratumoral heterogeneity of BRAF IHC could have an effect on the false negative rate, especially in small biopsied specimens. Therefore, more detailed evaluation criteria should be developed prior to the application of BRAF IHC in daily practice.

The initial test for HER2 status in breast and stomach cancer is IHC. For equivocal cases in HER2 IHC, an additional test, in situ hybridization, is recommended. In our previous meta-analysis, the concordance rate of the HER2 IHC 2+ subgroup between IHC and in situ hybridization was 0.393 (95 % CI 0.331–0.458) [24]. Also, in BRAF IHC, the concordance rate in the weakly positive subgroup was significantly lower than that in the remaining positive cases (0.386, 95 % CI 0.159–0.677 vs. 0.915, 95 % CI 0.861–0.950). According to our results, in weakly positive cases in BRAF IHC, an additional *BRAF* mutation analysis is recommended to confirm mutation status. When weakly positive cases were excluded, the overall concordance rate increased from 0.895 to 0.915. Similar to the recommended algorithm for HER IHC of breast and gastric cancers, the *BRAF* mutation test can be applied to weakly positive or equivocal cases but not to all cases.

There are a number of limitations to the current study. First, BRAF IHC using clone VE1 is specific for the *BRAF* V600E mutation. However, other *BRAF* mutations, such as V600 K, V600 M, V600R, V600D and V600G, could not be detected by BRAF IHC using VE1 in CRCs. Thus, false-negative cases in BRAF IHC using VE1 might have been caused by other *BRAF* mutations. Second, subgroup analysis for specimen type could not be performed due to insufficient information in eligible studies. To elucidate the usefulness of BRAF IHC based on specimen type, further studies are needed. Third, in

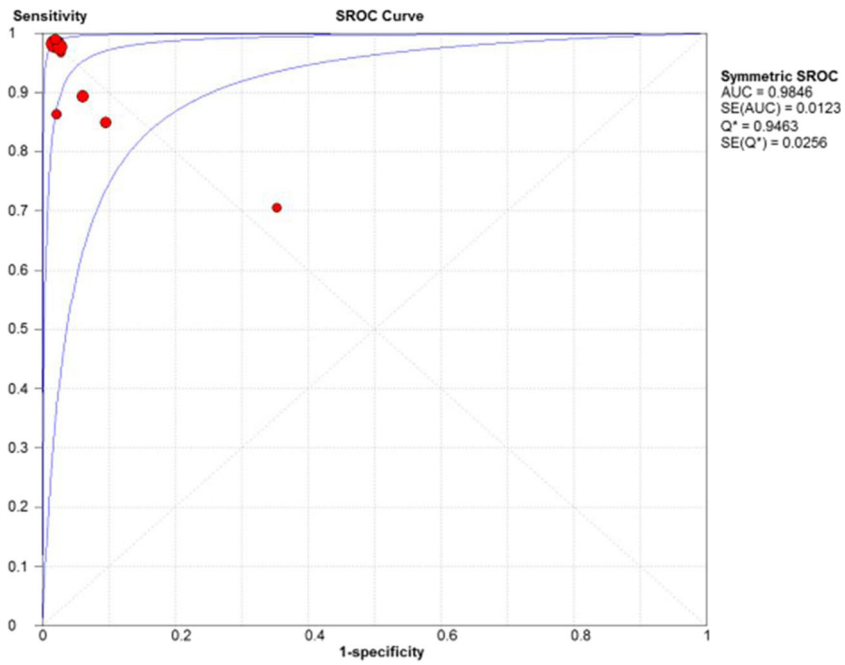

Fig. 3 The summary receiver operating characteristics (SROC) curve of BRAF immunohistochemistry in colorectal cancer

some patients with metastatic lesion, the specimen for evaluation of *BRAF* mutation can only be obtained from a metastatic lesion, not a primary lesion. However, the correlation of *BRAF* mutation between primary and metastatic lesions has not yet been fully elucidated in CRC [26]. Our meta-analysis was confined to primary lesions, regardless of metastasis. To elucidate the usefulness of BRAF IHC for *BRAF* mutation status, the concordance between *BRAF* mutation analysis and IHC in various metastatic foci should be evaluated. Last, our meta-analysis performed subgroup analysis for BRAF IHC weakly positive cases, and the concordance rate was lower than for overall positive cases. However, the number of included weakly positive cases was small (34), compared to the number of overall cases (n = 1021). As recommended in a previous study, an evaluation system, similar to the evaluation systems for HER2 status in breast and gastric cancer, would be useful for evaluation of *BRAF* mutation status [9, 27].

In conclusion, BRAF IHC using clone VE1 was strongly concordant with the *BRAF* mutation test in colorectal cancer. BRAF IHC in daily practice may come to replace $BRAF^{V600E}$ mutation analysis. However, further cumulative studies regarding a standardized method and evaluation system for BRAF IHC are needed before application in daily practice.

Compliance with Ethical Standards

Funding None.

Conflict of Interest The authors declare that they have no conflict of interest.

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