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ORIGINAL ARTICLE

A Multi-Institutional Validation of Gleason Score Derived from Tissue Microarray Cores

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Abstract

To test the agreement between high-grade PCa at RP and TMA, and the ability of TMA to predict BCR. Validation of concordance between tissue microarray (TMA) and radical prostatectomy (RP) high-grade prostate cancer (PCa) is crucial because latter determines the treated natural history of PCa. We hypothesized that TMA Gleason score is in agreement with RP pathology and capable of accurately predicting biochemical recurrence (BCR). Data were provided from a multi-institutional Canadian sample of 1333 TMA and RP specimens with complete clinicopathological data. First, rate of agreement between TMA and high-grade Gleason at RP or biopsy and RP was tested. Second, ability of RP, TMA and biopsy to predict BCR was compared. Multivariable (MVA) Cox regression models were fitted and BCR rates were illustrated with Kaplan-Meier plots. Agreement between RP and TMA and between RP and biopsy was 72.6% (95% CI:69.7–75.5) and 60.4% (95% CI:57.2–63.6), respectively. In MVA predicting BCR, the accuracy for RP, TMA and biopsy was 0.73, 0.72 and 0.68, respectively. TMA added discriminatory ability among exclusively low-grade Gleason RP patients ($p = 0.02$), but did not improve BCR discrimination in exclusive high-grade PCa RP patients ($p = 0.8$). TMA Gleason grade accurately reflects presence of high-grade Gleason in RP specimen, accurately predicts BCR rates after RP and improves prediction of BCR in low-grade Gleason patients at RP.

Keywords Radical prostatectomy · Prostate cancer · Gleason grade agreement · Biochemical recurrence · Validation

Sami-Ramzi Leyh-Bannurah and Dominique Trudel contributed equally to this work.

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Introduction

Tissue microarrays (TMA) represent the corner stone of large-scale analyses where molecular hypotheses are tested. [1] Despite the abundance of data and novel findings that were made thanks to TMA based analyses, TMAs were only tested once within a European cohort [2] with respect to their ability to agree with radical prostatectomy (RP) pathological high-grade prostate cancer (PCa) features that are recognized as established determinants of the treated natural history. [3, 4] Moreover, TMAs were not yet tested with respect to their ability to predict biochemical recurrence (BCR), based on Gleason patterns identified within the TMA core. It is of importance to confirm good concordance between TMA and RP high-grade PCa since high-grade PCa represents the gold standard determinant of the treated natural history of PCa. [5–7] For the same reason it is important to validate the ability of TMA core derived Gleason score to predict BCR, since the latter also reflects the treated natural history of PCa. [6, 8–14]

Based on these two considerations, we tested the agreement rate between high-grade PCa at RP and within the TMA core. Moreover, we also tested the ability of TMA core derived Gleason score to predict BCR. We also included biopsy derived high-grade PCa and Gleason score, as additional comparators. We hypothesized that TMA core derived Gleason is in agreement with RP pathology and additionally we posited that TMAs derived Gleason score is capable of accurately predicting BCR. For purpose of testing, we relied on a multi-institutional sample of 1516 TMA and RP specimens that represent the framework of the Canadian Prostate Cancer Biomarker Network (CPCBN) initiative of the Terry Fox Research Institute.

Material and Methods

Patient Selection

A total of 1516 Canadian prostate cancer patients were included in this study. Patients with missing biopsy, RP or TMA Gleason grade information were excluded ($n = 183$). All patients harboured non-metastatic PCa and all underwent a diagnostic transrectal prostate biopsy between 1990 and 2010. Patients underwent RP as primary treatment within 6 months after diagnosis.

Each RP sample was subjected to tissue procurement techniques for the purpose of TMA analyses. Clinical, pathological and biochemical recurrence (BCR) data were available for each of the 1333 patients. All subjects signed an informed consent to contribute to one of the participating biobanks and each institution's ethical board approved this study and institutional authorisation was obtained.

This study uses resources provided by the Canadian Prostate Cancer Biomarker Network's biobank funded by the Terry Fox Research Institute and managed and supervised by the Centre de recherche du centre hospitalier (CRCHUM).

Pathological Assessment and Tissue Microarray Format

Biopsy and RP Gleason grade assignments were performed according to the Gleason system effective at time of intervention and did not include tertiary grade. [15] Each paraffin embedded RP specimen was used to obtain one or multiple TMA cores for purpose of TMA analyses. The original paraffin blocks were retrieved from each tertiary care center archive, reviewed and tissue cores of 0.6 mm diameter were obtained from the zone of interest. [16]

Specifically, 4 μm TMA sections, colored with hematoxylin and eosin, were reviewed. The uropathologists also had access to the subsequent section, stained using the 34BE12-antibody to highlight basal membrane of benign glands. Briefly, sections were stained with the Benchmark XT autostainer (Ventana Medical System Inc.). Antigen retrieval was obtained using Cell Conditioning 1 (Ventana Medical System Inc., #950–124) for 60 min. Pre-diluted 34BE12 antibody (1:100) (Cederlane: CLSG36689–05) was manually added to the slides and incubated at 37 °C for 60 min. Reactions were performed using the UltraView universal DAB detection kit (Ventana Medical System Inc., #760–500). Counterstaining was achieved with hematoxylin and bluing reagent (Ventana Medical System Inc., #760–2021 and #760–2037). All sections were scanned using a VS-110 microscope with a 20 × 0,75NA objective and a resolution of 0,3225 μm (Olympus). Images were analyzed with the OlyVIA software (Olympus).

Dedicated uropathologists (D.T., M.L.) relied on up to 200× magnification to assign TMA Gleason patterns: primary, secondary and tertiary, if applicable. The uropathologists were unaware of clinical and pathological data. TMA cores were assessed without knowledge of PCa geographical location. Similarly to biopsy grading, Gleason score was assigned to each TMA core according to the most abundant (primary grade) and the highest (secondary grade) patterns. For each patient, according to clinical guidelines of biopsy interpretation, the core with the highest score was recorded. TMA categorization into high-grade was performed whenever pattern 4 or 5 was present.

Statistical Analyses

The endpoints of the study were two-fold: 1). the rate of agreement for presence of high-grade PCa at final pathology between either TMA and RP or biopsy and RP and 2). the ability to predict BCR with either TMA, RP or biopsy Gleason grade.

Agreement was quantified as percentage ranging from 0 to 100%.

Univariable, as well as multivariable Cox regression models (adjusted for age, PSA and clinical or pathological tumor stage, respectively) were fitted. The C-index was used to quantify the predictive ability of individual variables and of multivariable models. Examples of BCR rates were illustrated with Kaplan-Meier plots. To adjust for overfit bias and to simulate the analyses in 1000 similar cohorts of 1333 patients, we performed 1000 bootstrap re-samples. All tests were two-sided with p-values of 0.05 to indicate statistical significance. Analyses were performed using the statistical package for R (R foundation for Statistical Computing, version 3.2.2).

Results

Baseline, clinical and pathological characteristics of 1333 PCa patients are presented in Table 1. The median patient age was 62 years (interquartile range 57–66 years). Among all patients, the majority, 1013 patients (76.0%), had PSA values less than 10 ng/ml and 731 patients (54.8%) had clinical stage T1c. Biopsy demonstrated presence of high-grade PCa (Gleason score ≥ 7) in 626 patients (47.0%). At final pathology, 854 patients (64.1%) harboured organ confined disease (pT2) and 925 had high-grade PCa (69.4%). Overall 3736 TMA cores were available for analyses, with a median of three TMA samples per patient (range 1 to 8). In TMA cores, Gleason score ≥ 7 was identified in 898 patients (67.4%).

In the first part of our analyses, we tested the agreement between presence of high-grade PCa at RP and at TMA. For comparison purposes, we repeated the same analyses between RP and biopsy samples. With respect to RP and TMA, agreement of 72.6% was recorded (95% CI:69.7–75.5) and remained at 72.6% (95 CI:69.7–75.6), after 1000 bootstrap resamples. After stratification according to the number of TMA cores taken, into categories of ≤3 vs. ≥4, the rates of agreement were 72.1 and 75.2%, respectively ($p = 0.5$). These rates remained the same after bootstrapping. However, rates of agreement were only 53.7% and 65.8%, when either one or two TMA cores were obtained. Agreement of 60.4% (95% CI:57.2–63.6) was recorded, when RP was compared to biopsy and remained at 60.4% (95% CI:57.3–63.6) after bootstrapping. A detailed presentation of the individual Gleason scoring discrepancies in those patients with disagreement between high-grade prostate cancer at radical prostatectomy and low-grade prostate cancer at either TMA review or transrectal prostate biopsy are shown in Fig. 1. Within this subgroup, the majority, 92.8% and 94.0%, respectively, harboured a Gleason score 7 at RP, but low-grade prostate cancer at either TMA review or transrectal prostate biopsy.

In the second part of the analyses we examined the ability of RP derived Gleason grade to predict BCR relative to TMA core derived Gleason grade. As in the first part of the analyses, for comparison purposes, we also computed the ability of biopsy Gleason grade to predict BCR. After bootstrapping, RP Gleason grade demonstrated accuracy of 0.65 (95% CI:0.62–0.68) vs. 0.62 for TMA (95% CI:0.59–0.66) and 0.61 for biopsy (95% CI:0.57–0.64). When RP Gleason grade was combined with patient age, PSA and pathological stage, accuracy of 0.73 (95% CI:0.70–0.76) was recorded vs. 0.72 (95% CI:0.69–0.75), when TMA derived Gleason grade was combined with the same variables. For comparison purposes, when biopsy derived Gleason grade was combined with patient age, PSA and clinical stage, accuracy of 0.68 (95% CI:0.69–0.75) resulted.

We complemented our analyses with subgroup analyses to further illustrate the potential added benefit of TMA in patients, whose RP specimen contained either exclusively low-grade PCa or exclusively high-grade PCa. When TMA derived Gleason grade was considered among individuals with exclusively low-grade Gleason at RP, it added discriminatory ability regarding BCR ($p = 0.02$; Fig. 2). Conversely and expectedly, the TMA derived Gleason grade did not improve discrimination of BCR in individuals with exclusive high-grade PCa at RP ($p = 0.8$; Fig. 3).

Discussion

Our hypotheses stated that TMA core derived Gleason grading, contemporarily assigned by expert urogenital pathologists, accurately predicts the presence of high-grade Gleason at RP. Moreover, we postulated that TMA could predict BCR virtually as well as RP pathology. We tested these hypotheses by performing the analyses focusing on the presence of high-grade pathology at RP and TMA cores, as well as analyses focusing on BCR.

Our results were as follows. First, TMA cores were in close agreement with RP specimens regarding the presence of high-grade Gleason at RP (72.6%; 95% CI:69.7%–75.5%). This rate exceeded agreement recorded between biopsy and RP (60.4%; 95% CI:57.2%–63.6%). This clearly demonstrates that TMA core analysis is a better indicator of high-grade Gleason at RP than initial biopsy. Moreover, this result validates the ability of TMA to accurately identify high-grade foci of PCa that will likely determine the treated natural history of the disease.

Second, TMA core derived Gleason grade showed only marginally lower ability to predict BCR after RP than RP derived Gleason grade, in both univariable (0.62 vs. 0.65) and multivariable (0.72 vs. 0.73) analyses. This implies that TMA core derived Gleason grade very closely approximates that of RP. It also validates from another perspective, the ability of TMAs to identify those PCa foci that determine disease progression, defined as BCR.

Table 1 Baseline characteristics of 1333 patients diagnosed with prostate biopsy and treated with radical prostatectomy in the time period of 1990 to 2010, from whom TMA cores were obtained

characteristics		n / median	IQR / %
Age, year, median (IQR)		62	(57–66)
Year of surgery, time periods, n, %	1990–2000	276	20.7%
	2001–2004	528	39.6%
	2005–2010	529	39.7%
	total	1333	
PSA, ng/ml, categorized, n, %	≤ 4	188	14.1%
	>4 and < 10	825	61.9%
	<10	1013	76.0%
	10–20	250	18.8%
	> 20	70	5.3%
PSA, ng/ml, median (IQR)		6.7	(4.8–9.8)
Biopsy Gleason grade, n, %	3 + 3	707	53.0%
	3 + 4	369	27.7%
	4 + 3	134	10.1%
	Gleason score 8	81	6.1%
	Gleason score 9–10	42	3.2%
Clinical tumor stage, n, %	cT1c	731	54.8%
	cT2	76	5.7%
	cT2a	307	23.0%
	cT2b	122	9.2%
	≥ cT2c	54	4.1%
	unclassified	43	3.2%
Radical prostatectomy Gleason grade, n, %	3 + 3	408	30.6%
	3 + 4	550	41.3%
	4 + 3	196	14.7%
	Gleason score 8	100	7.5%
	Gleason score 9–10	79	5.9%
Pathlogical tumor stage, n, %	pT2	854	64.1%
	pT3a	343	25.7%
	pT3b	117	8.8%
	pT4	19	1.4%
Surgical margin, n, %	R0	872	65.4%
	R1	453	34.0%
	unclassified	8	0.6%
Lymph node invasion, n, %	N0	747	56.0%
	N1	38	2.9%
	unclassified	548	41.1%
TMA cores, median (IQR)		3	(2–3)
TMA cores, categorized, n, %	1	88	6.6%
	2	338	25.4%
	3	698	52.4%
	≥4	209	15.7%
TMA Gleason grade, n, %	3 + 3	435	32.6%
	3 + 4	439	32.9%
	4 + 3	201	15.1%
	Gleason score 8	242	18.2%
	Gleason score 9–10	16	1.2%

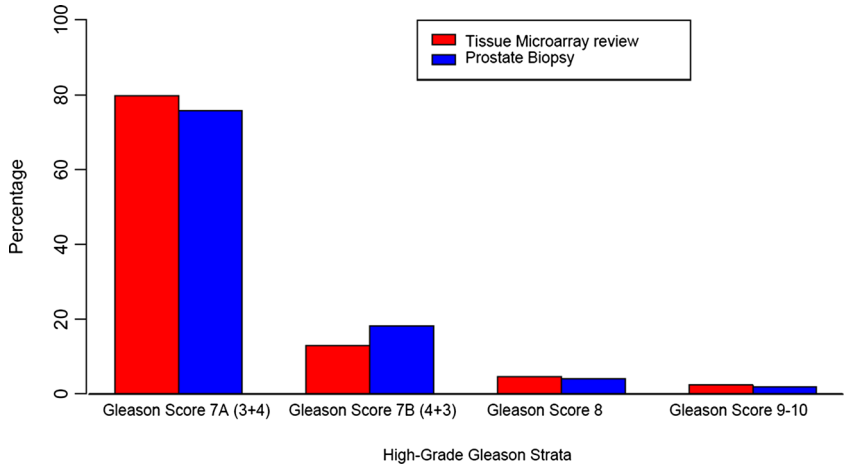

Fig. 1 Individual scoring discrepancies among those patients with high-grade prostate cancer at radical prostatectomy, stratified into Gleason scores 7A, 7B, 8 and 9–10, and low-grade prostate cancer at TMA review ($n = 253$) versus low-grade prostate cancer at transrectal prostate biopsy ($n = 366$)

Third, subgroup analyses performed in patients with exclusive low-grade PCa at RP also confirmed the added value of TMA derived tissue samples. Specifically, the TMA core derived Gleason grade improved the ability to predict BCR in individuals with exclusive low-grade Gleason sum at RP, in a statistically significant fashion ($p = 0.02$). Conversely and expectedly, as a means of validation, the TMA core obtained Gleason grade failed to improve the ability to predict BCR in individuals in whom the presence of high-grade Gleason was confirmed at RP.

Taken together, our results indicate that high-grade Gleason PCa assigned in TMA cores agrees with RP specimen. Moreover, Gleason grade assigned in TMA cores, either alone or in conjunction with several other variables, predicted BCR with virtually the same accuracy as RP Gleason grade, alone or in combination with the same additional variables. Last but not least, TMA core derived Gleason score improved discrimination of BCR among individuals with exclusively low-grade Gleason at RP.

To the best of our knowledge, our study represents the second formal validation of agreement of TMA derived high Gleason grade, relative to RP and first such endeavour in North American patients. [2] Moreover, our study represents the first validation of the ability of TMA core derived Gleason grade to predict BCR, relative to RP.

To date, only one group of European investigators performed a validation study of TMA specimens that included testing of agreement between RP and TMA core derived Gleason grades. Unlike our study, Wittschieber et al. only found moderate to even poor agreement, when Gleason grade was examined in 1912 TMA cores obtained from RP specimens in mostly 1:1 ratio. Several reasons may be proposed to explain the discrepancies. First, differences in PCa

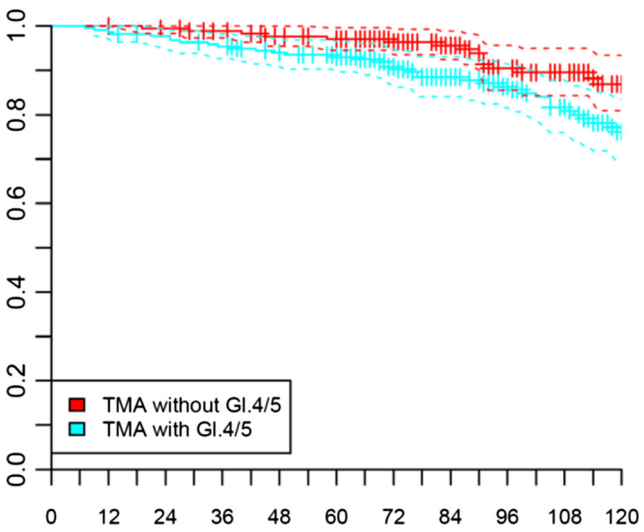

Fig. 2 Probability of biochemical recurrence free survival in patients with exclusive low-grade prostate cancer at radical prostatectomy (Gleason score 6) stratified according to presence or absence of high-grade prostate cancer within TMA cores ($p = 0.02$)

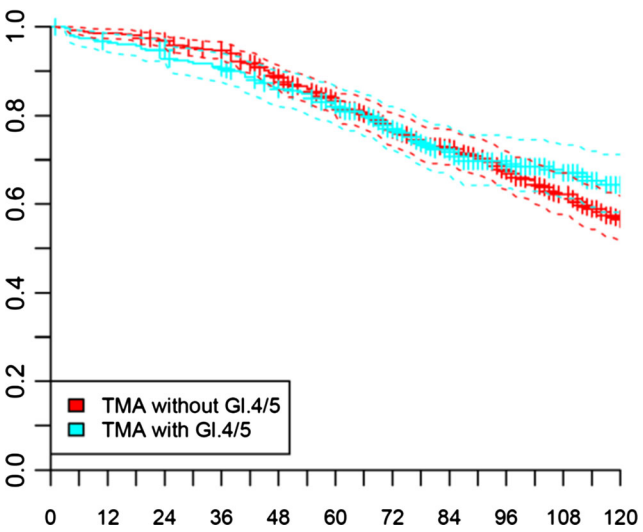

Fig. 3 Probability of biochemical recurrence free survival in patients with exclusive high-grade prostate cancer at radical prostatectomy (Gleason score 7 or greater) stratified according to presence or absence of high-grade prostate cancer within TMA cores ($p = 0.77$)

characteristics between European and North American patients may account for the differences. Moreover, unlike the previous study, we focused on high-grade Gleason since the objective of TMA cores is to reflect the natural history of PCa. The latter is determined by high-grade disease, and not by low-grade foci. In consequence, TMA cores aim to focus on high-grade PCa alone, when such cancer is present within RP specimen.

Second, differences in prevalence of low-grade PCa at RP between the current study and Wittschieber et al. may also have contributed to the observed discrepancy. Specifically, the rate of low-grade PCa was much higher in the study by Wittschieber et al. (46% vs. 31%). Presence of exclusive low-grade PCa in a high proportion of patients, as seen in the Wittschieber et al. study, renders the identification of high-grade PCa components impossible and thereby, by default, lowers the ability to predict high-grade PCa.

Third, the use of single TMA cores as done in the study by Wittschieber et al. vs. the use of multiple TMA cores with a median of three TMA cores in the current study, certainly contributed to better ability and thereby higher accuracy to identify high-grade PCa. It is possible that the detailed knowledge of clinical and biopsy characteristics prior to TMA procurement may help to further improve the recorded agreement. Moreover, the procurement of multiple TMA cores from each individual RP specimen (median of three in the current study) is in agreement with TMA core procurement recommendations, where the use, of three cores is endorsed. [17] Specifically, in the current study the use of three or fewer TMA cores did not result in a statistically significantly lower agreement rate relative to use of four or more TMA cores. However, the agreement rates recorded, when one or two TMA cores (respectively, 53.7 and 65.8%) were obtained, resulted in considerably lower agreement. This implies, that three TMA cores provide adequate tissue for accurate high-grade assignment. Moreover, it appears that the use of four or more TMA cores yields marginal, if any benefit when identification of high-grade PCa is targeted.

It is also important to note that unlike other studies, our analyses rest on TMA core Gleason assignment that was reviewed by two dedicated genitourinary pathologists. Moreover unlike in other analyses, we relied on bootstrapping to enhance the robustness of their statistical analyses by virtue of simulating analyses in 1000 cohorts of 1333 patients with varied clinical and pathological characteristics based on variable rates of resampling. Last but not least, relative paucity of data validating TMA core pathological findings call for more validation studies from other centers of excellence.

The clinical implication of our study is of utmost importance, since it validates the usefulness of TMA cores as proxies of high Gleason grade at RP and their ability to predict the natural history of treated PCa. In consequence, it appears valid to use material derived from TMA cores for other endpoints, such as biomarker or genetic marker analyses. [18–31] However, it is important to emphasize that TMA review is based on precisely pre-selected, optimized tissue samples of the index lesion of the RP resection sample with additional, dedicated genitourinary pathology workup. For that reason, it appears intuitive and consistent that our findings show TMA review to be more effective in identifying high-grade PCa in comparison to primary prostate core biopsy. Conversely, primary prostate core biopsy represents a systematic approach based on the whole prostate gland and is highly dependent on multiple clinical characteristics of the patient. [32] To overcome such limitations in daily clinical practice, systematic primary prostate core biopsy is increasingly replaced by targeted magnetic resonance ultrasound fusion prostate biopsy in primary and repeat biopsy setting, which represents a PCa index-lesion based approach similar to TMA review. [33]

Despite its strengths, the study is not devoid of limitations. First, the CPCBN database enrolled patients between 1990 and 2010. Approximately one fifth of the cohort was enrolled in the earliest time period of the study, 1990 to 2000. This may have affected Gleason grade assignment relative to contemporary methods, like all other studies that relied on pathological specimens dating from before 2005. [15] Second, RP specimen invariably did not include a tertiary Gleason grade. Third, the CPCBN sample consists of Canadian PCa patients. Their disease, diagnostic and therapeutic processes are reflective of Canadian health care, which is different from patients from the United States or Europe. Nonetheless, our findings need to be validated in similar cohorts from Europe and United States.

In summary, the results derived from our database demonstrate that TMA cores accurately reflect presence of high Gleason grade in RP specimen. Moreover, TMA core derived Gleason grade accurately predict BCR rates after RP. Finally, TMA cores derived Gleason grade improves prediction of BCR in patients with low Gleason grade at RP. Our findings should be validated in future cohorts.

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Authors' Contribution All authors of this research paper have directly participated in the planning, execution, or analysis of the study. All authors of this paper have read and approved the final version submitted. The authors listed below have made substantial contributions to the intellectual content of the paper in the various sections described below:

SR Leyh-Bannurah: data analysis, data management, manuscript writing.

D Trudel: data analysis, protocol development, manuscript writing.

M Latour: project development, manuscript editing.

E Zaffuto: manuscript editing, data analysis.
AA Grosset: manuscript editing, data collection.
C Tam: protocol development, project development, data collection.
V Ouellet: protocol development, project development, manuscript editing.
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N Fleshner: data analysis, protocol development, manuscript editing.
ME Gleave: protocol development, data collection.
AM Mes-Masson: protocol development, data collection.
F Saad: project development, data collection, data management, manuscript editing.
PI Karakiewicz: project development, data analysis, manuscript writing, manuscript editing.

Compliance with Ethical Standards

Conflict of Interest There are no conflicts of interest. The contents of this manuscript have not been copyrighted or published previously. The contents of this manuscript are not under consideration for publication elsewhere.

Ethical Standards Informed written consent was obtained and ethical standards were adhered to.

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