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Case Report

Poorly differentiated medullary carcinoma of the colon with an unusual phenotypic profile mimicking high grade large cell lymphoma – a unique case report and review of the literature

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Abstract: Medullary carcinoma (MC) of the colon and rectum is a rare entity, accounting for less than 0.1% of colonic adenocarcinoma that poses a diagnostic challenge for the practicing pathologist. Poorly differentiated or undifferentiated MC with an unusual histological appearance and immunoprofile in addition to heavy lymphoid infiltrate could make it problematic when differentiating it from a high grade lymphoma, in particular anaplastic large B- or T-cell lymphoma, plasmablastic lymphoma, and other undifferentiated neoplasms. Here we reported a unique case of an 81 y/o woman presenting with a 7.0 cm colon mass detected by computed tomography (CT) scan. A partial transverse and ileum resection with appendectomy were performed. Microscopic examination revealed sheets of large, pleomorphic, mitotically-active cells with abundant eosinophilic cytoplasm and multiple prominent nucleoli, growing with a pushing border and poor glandular formation in a background of intratumoral lymphocytes. The neoplastic cells were only focally positive for keratins (<10%); diffusely and strongly positive for vimentin and CD10 with high proliferative index (Ki-67, 90%). The tumor cells were also aberrantly positive for CD30, CD79a and CD43 (diffusely or focally), resulting in a diagnostic dilemma between colonic MC and high grade lymphoma. Careful examination and additional immunohistochemical stains performed proved there was no evidence of T or B-cell lymphoma, melanoma, or other types of primary colon or metastatic carcinomas. This case highlights the difficulty in distinguishing a high grade lymphoma and poorly differentiated colonic MC, and, also the aberrant expression of CD10 and a significant loss of pancytokeratin could result in a diagnostic pitfall.

Keywords: Medullary carcinoma, colorectal, lymphoma, microsatellite instability

Clinical history

We present a review case from a patient of Moffitt Cancer Center who was being evaluated at the institution for further management and workup. The patient is an 81 year-old Caucasian female with a past medical history of hyperlipidemia, hypothyroidism, and hypercoagulable state with deep vein thrombosis. Moreover, she was diagnosed with melanoma of the anterior chest wall, which was treated with wide local excision whose margins were clear. No chemotherapy or biotherapy was documented. She presented to a local hospital with syncope and evidence of a lower gastrointestinal bleed in June of 2012, and further CT scan of CT neck, thorax, abdomen, and pelvis revealed a 7.0 cm colonic mass. A metastatic melanoma was suspected. A partial transverse and ileum resection with appendectomy was subsequently performed. No additional chemotherapy has been administrated. Seven months later, an imaging study showed a reduced size of her lymphadenopathy as well as no evidence of recurrent disease in the colon or rectum.

Pathological findings

Gross description

Based on the provided outside pathology report, the specimen included the partial colec-

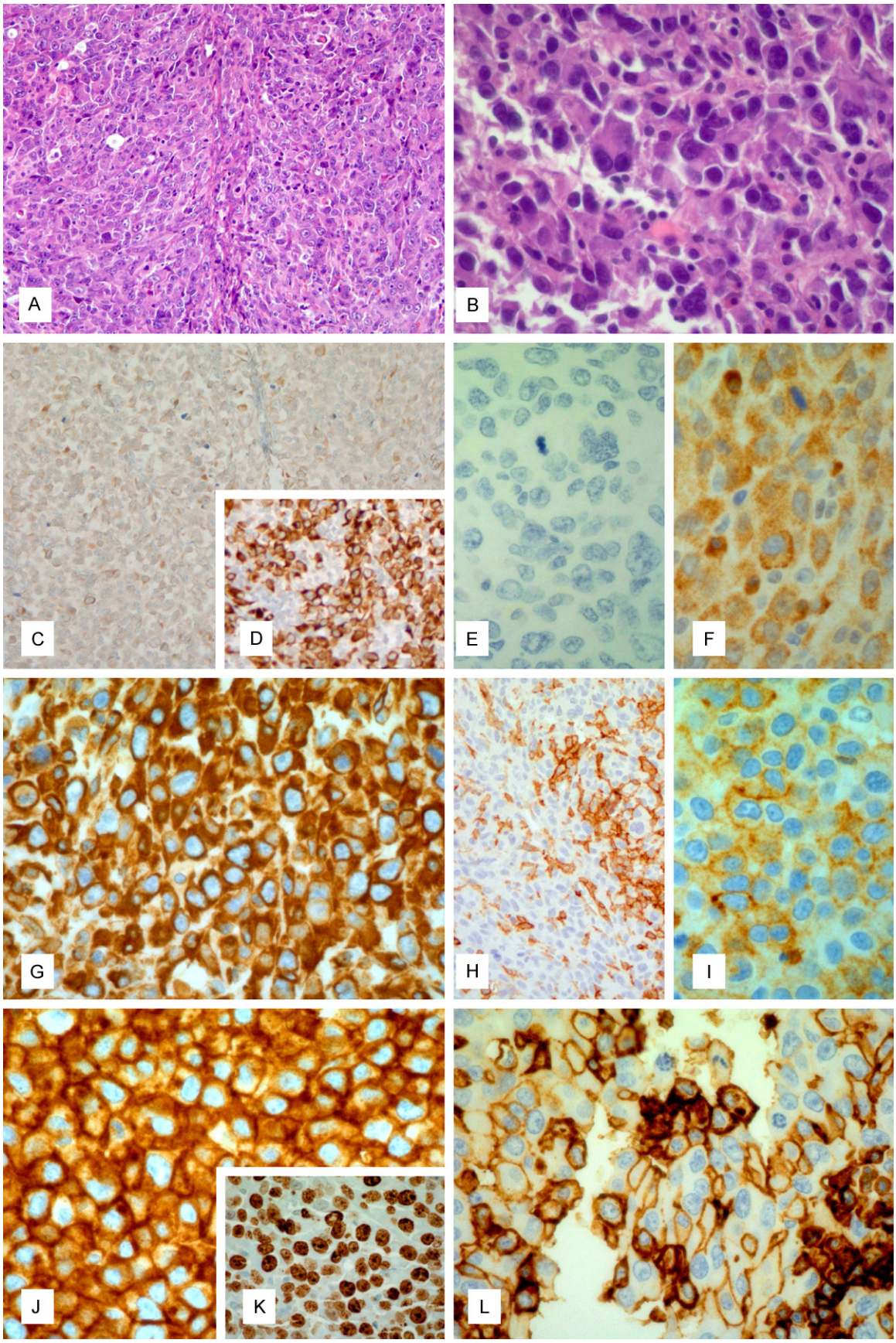
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Figure 1. (A and B) Sections of colonic MC showed a pushing border infiltrative pattern and a focal anaplastic, discohesive appearance (H&E, x 200 and x 600, respectively). (C and D) Cam5.2 stain was largely negative in tumor but focally strong immunoreactivity (10% of tumor cells) (immunoperoxidase x 200). A CD20 stain was negative (E) and CD79a stain (F) showed brushing cytoplasmic positivity (immunoperoxidase, x 600). (G) Vimentin was strongly positive for the tumor cells (immunoperoxidase, x 600). (H) CD3 stain highlights background small lymphocytes (immunoperoxidase, x 200). (I) CD30 stain demonstrated patchy weak reactivity in some large cells (immunoperoxidase, x 600). (J and K) Strong CD10 immunoreaction was noted in all tumor cells (J) with high proliferation rate highlighted by Ki67 (K) (immunoperoxidase, x 600). (L) CD45 stain was positive in small to large reactive lymphohistiocytic cells but essentially negative for the tumor cells (Immunoperoxidase, x 600).

tomy (22 cm in length x 6.0 cm in internal circumference), partial ileum (8.0 and 9.0 cm in lengths), appendectomy (6.0 cm in length) as well as a 7.0 cm centrally ulcerated, friable, fungating, circumferential mass of colon, which was widely clear of the proximal and distal margins. The mesenteric margin was also uninvolved by tumor, but focally involved the subserosal adipose tissue. A total of twelve (12) mesenteric lymph nodes were successfully retrieved for microscopic examination and entirely submitted along with adequate sections of the lesion itself and appropriate margins.

Histology

Routine hematoxylin and eosin (H&E) sections of formalin fixed, paraffin embedded tissue blocks of the submitted tissue demonstrated sheets of well-circumscribed, moderately to severely pleomorphic, hyperchromatic cells with medium-to-large sized nuclei with a vesicular chromatin pattern, prominent nucleoli, growing with an expansile pattern but confined with a pushing border. The neoplastic cells contained moderate to abundant eosinophilic cytoplasm, with a noticeable intratumoral as well as peritumoral small lymphocytic infiltrates and increased histiocytes/macrophages (**Figure 1A**). Anaplastic forms with giant, hyperchromatic, "horse-shoe-shaped" or smudged nuclei were focally present. Bi- and multinucleation were identified. Brisk mitoses (1-4/HPF) and multifocal "dirty necroses" were observed. However, there was no evidence of glandular or tubular formation. Focally, the tumor cells displayed dis-cohesive or individual arrangement (**Figure 1B**). The tumor cells infiltrated through the muscularis propria and involved the adjacent subserosal fat, which, therefore, designated the pathologic stage as pT3. Metastatic involvement of 3 out of the 12 mesenteric lymph nodes with no available information of distant metastasis further characterized the pathologic stage as pT3, pN1b, and pMX. Furthermore, vascular and perineural invasion were present. The submitted appendix was uninvolved by tumor and contained no specific pathological findings.

Immunohistochemistry

As with many, if not all, cases of poorly-differentiated or undifferentiated tumors of uncertain lineage, a battery of immunohistochemical stains must be performed in order to further classify and subclassify the lesion. Per outside contributor, the following immunohistochemical stains were performed. The stained slides were available for interpretation. The neoplastic cells were largely negative for many carcinoma markers including CK7, CK20, DOG-1, and RCC but focally positive for cytokeratin (EMA and AE1/AE3), constituting 10% of the total neoplasmic cells (**Figure 1C** and **1D**). They were strongly positive for vimentin (**Figure 1G**) and CD10 (**Figure 1J**) focally and weakly positive for CD30 (**Figure 1I**). These cells were also negative for plasma cell markers (CD138 and MUM-1), melanoma markers (HMB-45, S100, and Melan A), muscular markers (myosin and desmin), dendritic cell markers (CD1a and CD35), hematopoietic markers (CD117, CD34, CD71 and MPO) and ALK-1. An in-situ hybridization study using an Epstein-Barr-encoded RNA probe showed no abnormal signal. The intratumoral and peritumoral lymphocytes showed patchy positivity for CD45 (**Figure 1L**), CD3 (**Figure 1H**), CD4, CD5, CD7 (partial) and CD8 and negative for CD20 (**Figure 1E**) and PAX-5. However, CD79a stain showed patchy cytoplasmic positivity (**Figure 1F**).

Upon review of the H&E and the outside immunohistochemical slides, additional immunohistochemical stains were performed, specifically, mucicarmine, MUC-1, MUC-2, MSH2, MSH6, PMS2, MLH1, Cam5.2, CD56, chromogranin, synaptophysin, Ki-67, CDX2, and calretinin. The

Table 1. Key Immunochemistry Study Implicated in the Case Diagnosis

Name of Antibody	Company	Antibody Clone Name	Results (pos or neg, % of total cells)
CAM5.2	Becton Dickinson	CAM5.2	Positive (10%)
CK7	DAKO	OV-TL-12/30	Negative (100%)
CK20	DAKO	KS20.8	Negative (100%)
EMA	Ventana Medical Systems	E29	Weak positive (10%)
ALK	Ventana Medical Systems	ALK01	Negative (100%)
CD3	Ventana Medical Systems	2GV6	T lymphocyte Positive
CD4	Ventana Medical Systems	SP35	Subset lymphocytes and histiocyte positive
CD8	LEICA	1A5	Subset lymphocytes positive
CD10	LEICA	56C6	Lymphocytes positive (90%)
CD20	Ventana Medical Systems	L26	Lymphocytes negative
CD30	Cell Marque	BER-H2	Positive (15%)
CD45	Ventana Medical Systems	RP2/18	Lymphocytes/granulocytes positive
CD56	LEICA	CD564-1B6	Negative
CD79a	Ventana Medical Systems	SP18	Weakly positive (15%)
MUM1	Cell Marque	MRQ-8	Negative (100%)
MLH1	Ventana Medical Systems	G168.728	Positive (nuclear, 100%)
MSH2	Ventana Medical Systems	62191129	Positive (nuclear, 100%)
MSH6	Cell Marque	44	Positive (nuclear, 100%)
PMS2	BIOGENEX	ERPR3947	Positive (nuclear, 100%)
CDX-2	BIOGENEX	CDX2-88	Negative (100%)
MUC1	CM	MKQ-17	Negative (100%)
MUC2	Ventana Medical Systems	MRQ-18	Negative (100%)
Vimentin	Ventana Medical Systems	3B4	Positive (100%)
Chromogranin/Synaptophysin	Cell Marque	LK2H10	Negative (100%)
Melan A	Ventana Medical Systems	A103	Negative (100%)
HMB-45	NC	HMB	Negative (100%)
Ki67	Ventana Medical Systems	30-9	Positive (90% of neoplastic cells)

lesional cells showed focal positivity for Cam5.2 but strong nuclear positivity for MLH1, MSH2, MSH6, and PMS2. Ki-67 staining showed a high proliferative index of 90% (**Figure 1K**) via manual quantitative morphometric analysis. The remaining additional stains performed were interpreted as negative. The key findings of the immunohistochemical stains from both the outside contributor and MCC are summarized in **Table 1**. The overall features support a diagnosis of MC of the colon.

Molecular studies

The working diagnosis of the outside institution prior to review at the Moffitt Cancer Center was "Anaplastic Large Cell Neoplasm, favor Anaplastic Variant of Diffuse Large B-Cell Lymphoma", which prompted the contributor to submit a tissue block for B-cell gene rearrangement studies to a reference laboratory. A clonal rearrangement of T-cell receptor or B-cell immunoglobin heavy chain (IGH) amplification was not detected.

Discussion

Medullary carcinoma of the colon and rectum (MC) is now widely recognized as a subtype of colorectal adenocarcinoma with its own distinct clinical, histological, immunophenotypical, and prognostic aspects. Previously referred to as "large cell adenocarcinoma with minimal differentiation", the World Health Organization (WHO) now categorizes MC as a rare variant of colorectal adenocarcinoma composed of sheets of malignant cells with vesicular nuclei, prominent nucleoli and abundant eosinophilic cytoplasm with prominent infiltration by intraepithelial lymphocytes [1]. MC is an exceedingly rare entity, and has been reported to only comprise 0.03% of all sporadic colorectal adenocarcinoma cases [2]. MC of the colon has a predilection for elderly females with a mean age of

69.3 years [2], Caucasian patients [2], generally occurs on the right side (ascending colon and rectum) [3, 4], and is associated with a better prognosis owing to its lower pathologic stage and presence of local nodal metastasis at time of presentation as compared to the other poorly-differentiated adenocarcinomas (PDA) or undifferentiated adenocarcinomas (UDA) [1, 2, 5]. The majority of MC cases are associated with germline mutations in the DNA mismatch repair genes, causing microsatellite instability in up to 60% of all cases [1]. However, our case showed all intact nuclear immunostaining pattern, indicating no loss MLH1, MSH2, MSH6, and PMS2 markers, and, thus, was a microsatellite stable tumor.

Our case highlights the difficulty in interpreting immunohistochemical stains in the face of poorly-differentiated or undifferentiated neoplasms of the colon and rectum. One must be careful in distinguishing true positive staining within the lesional cells as opposed to surrounding non-neoplastic cells such as macrophages, stromal cells, or, in our case, the intratumoral T-lymphocytes and B-lymphocytes. This was shown by the focal positivity for CD45 in non-neoplastic background cells as well as nonspecific cytoplasmic staining of CD79a, resulting in a misinterpretation as "Anaplastic Large Cell Neoplasm, favor Anaplastic Variant of Diffuse Large B-Cell Lymphoma". For CD20 negative and CD79a positive neoplastic cells with anaplastic morphology, a differential diagnosis of plasmablastic lymphoma of GI was included. However, negative positivity for light chain clonality, CD138 and MUM1 immunohistochemistry, and EBER in-situ hybridization excluded the diagnosis of plasmablastic lymphoma. Intratumoral and peritumoral Crohn's-like lymphoid aggregates are a distinct morphological feature of MC of the colon which represents a host response to the tumor and may serve to improve survival rates, which were observed in our case and highlighted by immunohistochemical stains with several T-cell markers (CD3, CD4, CD5, CD7, CD8, and CD43) [6]. A 3-tiered classification scheme has been proposed to categorize the Crohn-like disease lymphoid response, and has been shown to correlate directly with the incidence of lymph node metastasis and improved overall survival [6]. It has been reported, however, that a Crohn-like disease lymphoid response is associated with medullary-type histology than peripheral infiltration of lymphocytes, and up to 30% of MCs may not have any type of lymphocytic infiltration [4].

CDX2 is a gene responsible for producing a intestinal-transcription factor expressed in the nuclei of intestinal epithelial cells. It is considered to be a sensitive and specific marker of intestinal differentiation [5]. Winn et. al and Hinoi et. al both showed significantly lower expression of CDX2 in MC compared to PDA, suggesting loss of expression of intestinal differentation, and, our case did not express CDX2 [7, 8]. Additionally, other markers of intestinal differentiation include MUC1, MUC2, and TFF3. MUC1 is a non-secretory glycoprotein located along the apical membrane of colonic epithelium [5] while MUC2 is the main mucin secreted by goblet cells spaced among intestinal columnar epithelium [5]. TFF3, or intestinal trefoil factor, is a component of normal intestinal goblet cells, and considered part of the pathway of epithelial regeneration [5]. Though our institution did not evaluate the presence of TFF3 in the specimen, one can see the potential pitfall of diagnosing MC when the markers of intestinal differentation (CDX2, MUC1, MUC2, and mucicarmine) were all negative. MUC1, MUC2, and TFF3 has been reported to be present in 67%, 60%, and 53% of MC, respectively, indicating that most MCs retain their intestinal origins despite their undifferentiated appearance [5]. Combined with the only focal positivity for pankeratin (AE1/AE3), this can create a diagnostic dilemma for the practicing pathologist who has never encountered such a rare entity. It is well known that vimentin can be expressed in some poorly differentiated carcinomas in addition to lymphoma and other mesenchymal tumors, which also occurred in our case. Nevertheless, the characteristic histological appearance, presence of intratumoral and peritumoral lymphocytic infiltrates, undeniably positive microsatellite-stability markers, and lack of a clonal population of lymphocytes makes MC a much more likely diagnosis than a high grade lymphoma.

Interestingly, our case demonstrated strong positivity for CD10 within the lesional cells and not within the surrounding stromal cells. CD10 is a 90-100 kDa cell membrane metallopeptidase expressed by early B, pro-B and pre-B

lymphocytes as well as germinal centers and non-hematopoietic tissue such as fibroblasts [9]. CD10 overexpression and its correlation to clinical outcome has been reported in numerous neoplasms [9-11], such as breast carcinoma and gynecologic tumors, but not mentioned in MC. Data regarding the correlation of stromal and tumoral CD10 expression and tumor progression and clinical outcomes is scant and conflicting. CD10 expression in neoplastic cells may contribute to apoptosis and proliferation while its expression in stromal cells may be associated with tumor and CD10 in invasive breast carcinoma [9, 10]. However, Oba et. Al demonstrated in a study of 64 cases that the majority of malignant melanomas (53%, 34/64) expressed CD10 in contrast to only 10%, or 4/64 of melanocytic nevi [11]. However, the investigators showed that stromal expression of CD10 was not (p=0.16) associated with tumor progression, and, thus overall patient survival outcomes [11]. In a separate study, Vo et. Al found that the majority of patients with invasive breast carcinoma (53.4%, 39/73) had aberrant expression of CD10, and, of those 39 cases, 41% (16/39) eventually recurred while 66.7% (26/39) had regional lymph node metastasis [12]. Stromal CD10 expression correlated with tumor size (p=0.009), reigonal lymph node status (p=0.028), estrogen receptor status (p=0.013), Ki67 index (p=0.002), and disease-free survival (p=0.008), however, it failed to show statistical signifiance with age interval, histologic grade, histologic type, PgR status, and distant metastasis (all p>0.05). Tumoral expression of CD10 was absent in one of our medullary carcinomas but strongly positive in the other (**Figure 1J**), thus, a larger sample size and further studies would be required to make significant conclusions. Neoplastic cells occupy a complex surrounding stromal microenvironment that consists of a dynamic interaction between cytokines, interleukins, extracellular matrix, and tumor cells, and perhaps it is this complexity that determines the role of CD10 in tumor progression. Nevertheless, strong CD10 immunostaining may serve to further confuse or falsely reassure the practicing pathologist, when, in reality, it may represent an underlying transformation or progression of MC. A relative short follow up (7 months after diagnosis) prevented us from predicting the long term outcome of the patient. In our case, in particular, when the patient had a history of malignant melanoma, CD10 positivity in neoplastic cells triggered additional stainings (S100, HMB45 and Melan A) to exclude metastatic carcinoma.

Conclusion

MC of the colon and rectum has now come into the spotlight as a distinct but extremely rare variant of adenocarcinoma with unique clinical, histological, immunophenotypical, and prognostic characteristics. Accurate diagnosis is important as it confers a more favorable prognosis due to its underlying microsatellite stability mutations. Poorly differentiated MC without microsatellite stability mutations could occur. A distinguishable genetic background without germline mutations in the DNA mismatch repair genes, aberrant phenotyping (CD10 positivity, vimentin positivity, and significant loss of cytokeratin expression) and a high proliferation index might predict a different clinical outcome, which warrants a close follow-up. Further investigation into overexpression of CD10 is needed to elucidate its role in the molecular basis for the clinical behavior of MC of colon as well as the other solid tumors.

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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