Table 1 Sensitivity and specificity of Ki-67 proliferative index (PI) at progressively enlarging hotspots in comparison to the minimum recommended (gold standard) hotspot size of 1000 cells

Hotspot size	*n*	True high Ki-67 PI	True low Ki-67 PI	False high Ki-67 PI	False low Ki-67 PI	Sensitivity	Specificity
1000−2000	32	20	12	–	–	–	–
2001−4000	32	19	12	0	1	0.95	100
4001−8000	32	19	12	0	1	0.95	100
8001−16,000	32	19	12	0	1	0.95	100
16,001−32,000	31	17	11	0	3	0.85	100
>32,000	29	15	9	0	5	0.75	100

which may contribute to poor interobserver correlation, albeit one unique to groups/laboratories utilising digital systems for such evaluations.

This is a small study and requires verification in a larger cohort and ideally against outcome data in order to determine the optimal counting area with the strongest correlation to clinical outcomes. However, until such studies are available it seems prudent for those reporting Ki-67 PI using digital technology to limit their maximum hotspot size; PathWest will adopt a 16,000 cell hotspot size maximum in evaluations going forward. There is also a need to consider an upper cell enumeration limit in any future international guidelines for Ki-67 PI evaluation in IBC.

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1. Inwald EC, Klinkhammer-Schalke M, Hofstädter F, *et al*. Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry. *Breast Cancer Res Treat* 2013; 139: 539−52.
2. Fischer B, Jeong JH, Dignam J, *et al*. Findings from recent National surgical adjuvant breast and bowel project adjuvant studies in stage I breast cancer. *J Natl Cancer Inst Monogr* 2001; 30: 62−6.
3. Coates AS, Winer EP, Goldhirsch A, *et al*. Tailoring therapies−improving the management of early breast cancer: St Gallen International expert consensus on the primary therapy of early breast cancer 2015. *Ann Oncol* 2015; 26: 1533−46.
4. Chen X, He C, Han D, *et al*. The predictive value of Ki-67 before neoadjuvant chemotherapy for breast cancer: a systematic review and meta-analysis. *Future Oncol* 2017; 13: 843−57.
5. Harvey J, Thomas C, Wood B, *et al*. Practical issues concerning the implementation of Ki-67 proliferative index measurement in breast cancer reporting. *Pathology* 2015; 47: 13−20.
6. Dowsett M, Nielsen TO, A'Hern R, *et al*. Assessment of Ki-67 in breast cancer: recommendations from the International Ki-67 in breast cancer working group. *J Natl Cancer Inst* 2011; 103: 1656−64.
7. Honma N, Horii R, Iwase T, *et al*. Ki-67 evaluation at the hottest spot predicts clinical outcome of patients with hormone receptor-positive/HER2-negative breast cancer treated with adjuvant tamoxifen monotherapy. *Breast Cancer* 2015; 22: 71−8.

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Clear cell sarcoma of the soft palate mimicking unclassified melanoma

Sir,

Clear cell sarcoma (CCS) of soft tissue is a rare sarcoma that shares overlapping histogenic, morphologic and immunophenotypic features with melanoma. Soft tissue CCS typically arises in lower extremity tendons and aponeuroses of young adults, with a female predominance. It has very rarely been reported to involve the gastrointestinal tract or head and neck region. Herein, we describe CCS of the soft palate.

A 27-year-old man with no significant past medical history presented with a 4 cm lesion on the palate. Over the past three years, the patient had reportedly presented to the clinic for palatal ulcerations on multiple occasions. On examination, the lesion was ill-defined and erythematous, with a friable centre, superficial erosions, and irregular, raised edges (Fig. 1A). Two successive biopsies were performed, and pathology obtained at the outside institution described an unclassifiable melanocytic tumour in both cases. We received the second biopsy in consultation. Histological sections demonstrated a small sample of a larger tumour, comprised of nests of oval to epithelioid cells which appeared to abut the overlying epithelium (Fig. 1B). Mitotic figures were readily identifiable. Immunohistochemical staining showed diffuse MelanA positivity and clonal loss of p16 protein expression. A diagnosis of unclassified melanoma was rendered. Radiological staging was performed using magnetic resonance imaging (MRI) and positron emission tomography (PET) scanning. The lesion was not visible on MRI, and palatal osteolysis was not identified. Full body PET scan was positive for hypermetabolic activity in the draining jugular-carotid lymph nodes but was otherwise negative.

Given the unusual clinical and histopathological features of this case, as well as the advanced stage of disease at presentation, RNA-sequencing was performed to guide management. The results were negative for hotspot mutations in *BRAF, NRAS*, and *CKIT*, but did identify an *EWSR1-ATF1* fusion. The diagnosis was revised from unclassified melanoma to clear cell sarcoma of the palate. The

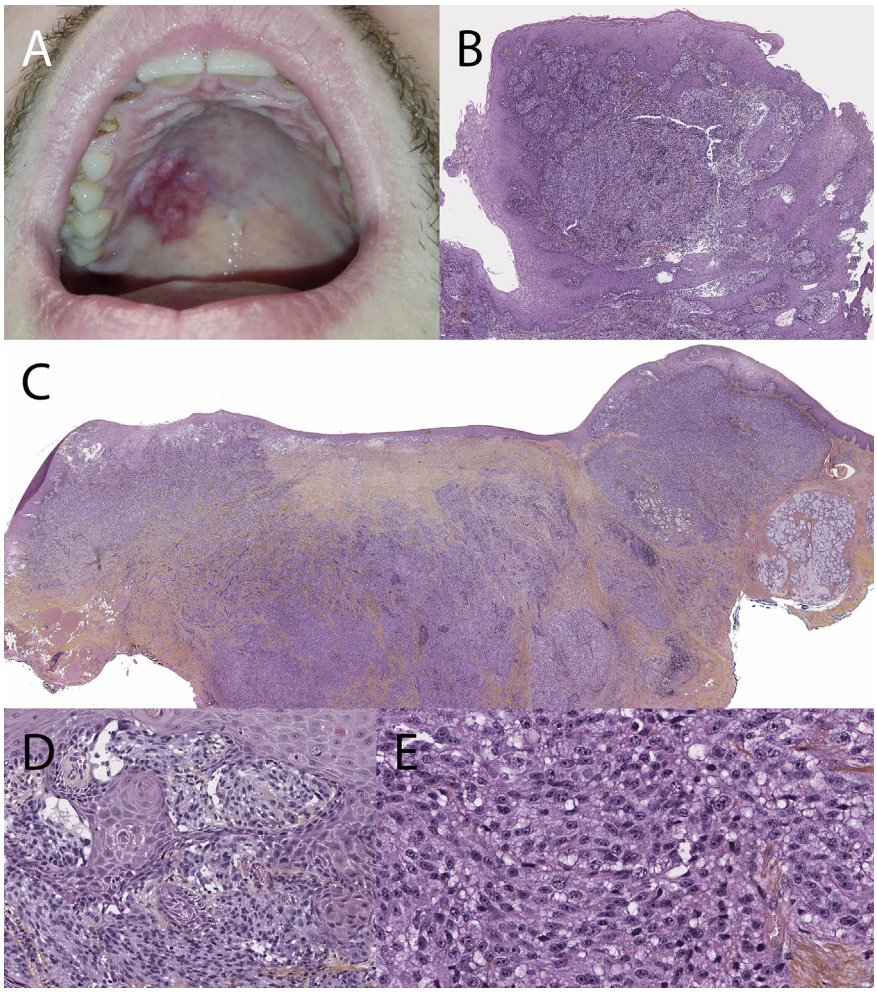

Fig. 1 Clinical and pathological presentation: (A) ulcerated and budding lesion of the right palate, (B) low power view of initial biopsy, (C) low power view of tumour resection with dense cellular sheets and nests extending deeply, (D) close-up view of C underscoring the confluent peri-mucosal nests, (E) nest of large atypical cells with an oval hyperchromatic nuclei with often a prominent nucleoli and a granular eosinophilic or clear cytoplasm.

wide surgical resection was modified to include subperiosteal cleavage followed by milling of the palatal arch without immediate reconstruction. The resection specimen was pathologically similar to the biopsy specimen and additionally showed expansile nests and cords of spindled and epithelioid tumour cells extending throughout the submucosa, with scarred ulceration of overlying epithelium. There was no appreciable maturation with descent of tumour (Fig. 1C). Necrosis was not identified. Rare clusters and isolated tumour cells extended into the epithelium, although true pagetoid spread was absent (Fig. 1D). Tumour cells displayed pale eosinophilic and vacuolated cytoplasm without pigmentation, and were uniform, with enlarged oval nuclei with open chromatin and prominent nucleoli. Wreath-like multinucleation was not identified, and 12 mitosis per 10 high power fields were counted (Fig. 1E). Scattered lymphoplasmacytic cell aggregates were noted; intra-vascular invasion was absent. Immunohistochemistry confirmed melanocytic phenotype, with diffuse positivity of MelanA, HMB45, MiTF and Sox10 (Fig. 2). Completion maxillectomy was refused by the patient. Fractionated radiotherapy [50 gy (25×2 Gy)] was delivered to the tumour bed and cervical lymph nodes. In the 6 months follow-up available, no relapse has been observed.

The current case is unique in its clinical presentation, histomorphological features, and immunophenotypical findings. Presentation of CCS in the oral cavity is extremely rare. Table 1 summarises the clinical, morphological, immunohistochemical, and molecular features of oral CCS previously reported in the literature, including the current case.[1–6] Of the six other published cases, three were located in the tongue, one in the parapharyngeal space, one in the submucosa of the jaw with bony involvement, and one in the lip. These tumours were deeply seated in all but one case; the parapharyngeal case was superficial and was associated with multiple shallow ulcers and a rolled border, as seen in the current case.

Five of six previously reported cases of oral CCS showed classic histopathological features, including dense fascicles and sheets of large spindled to epithelioid cells in a fibrous pseudo-encapsulated nodule. Nesting of tumour cells at the epithelial-mucosal interface with focal intra-epithelial spread, as observed in the current case, was not described. The close association of CCS with epidermis has been reported to rarely occur in cutaneous soft tissue tumours[7] as dense dermal spindle cell fascicles that abut the epidermis and mimic junctional melanocytic nests. In one case, rare scattered junctional melanocytes were also noted.[8]

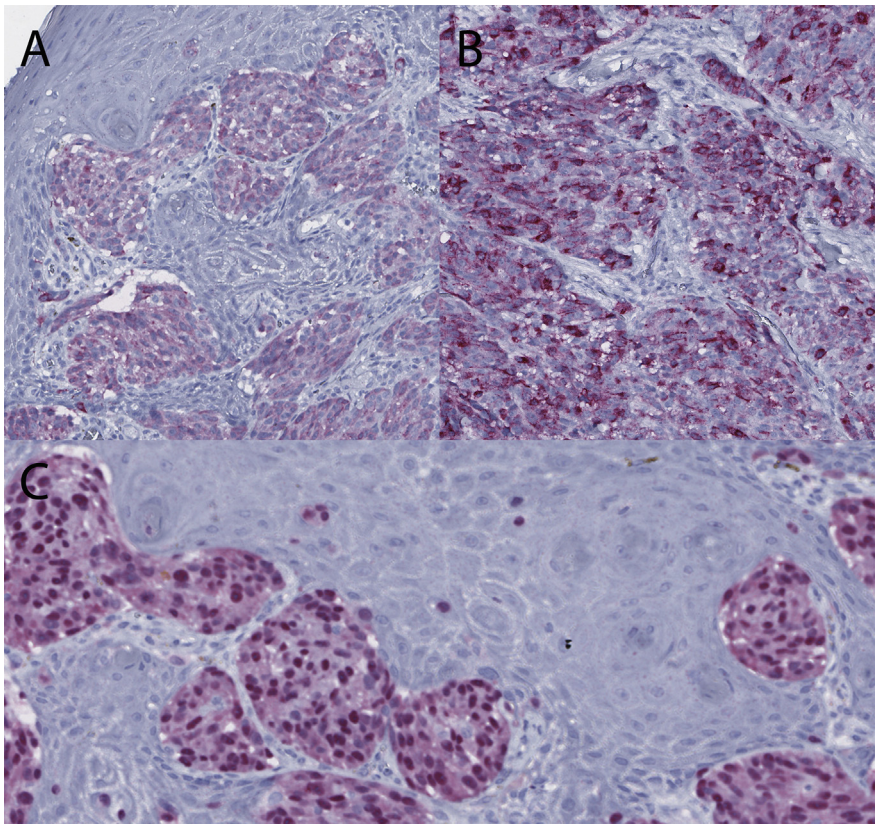

Fig. 2 Immunohistochemical stains. MelanA (A), HMB45 (B) and MiTF (C) were strongly and diffusely positive in the tumour cells including the perimucosal nests (A,C) with a few isolated junctional clusters or isolated intramucosal cells (C).

Of six reported oral cavity associated CCS, one case, originating in the tongue, displayed morphological features of 'CCS-like tumour of the gastrointestinal tract' or gastrointestinal neuroectodermal tumour (GNET). This variant, described in 2003, has *EWSR1-ATF1* or *EWSR1-CREB1* fusions, shows relatively less melanocytic differentiation, a pseudoalveolar pattern, necrosis, and scattered osteoclast-like multinucleated giant cells.[9] They express S100-protein, Sox10 and vimentin, but are negative for other melanocytic markers such as HMB45 and MelanA.[10] The other reported cases of oral CCS focally expressed S100 and HMB45. However, MelanA was negative in three out of four cases.

Clear cell sarcoma is characterised by *EWSR1* gene rearrangements. Translocation of t (12; 22) (q13; q13) resulting in fusion of *EWSR1* and *ATF1* genes is identified in over 90% of cases by polymerase chain reaction (PCR) or fluorescence *in situ* hybridisation (FISH) studies, and in 70% of cases by conventional cytogenetics. Importantly, fusion of *EWSR1* and *ATF1* has not been identified in melanoma. Due to the histological and immunophenotypical overlap between melanoma and CCS, molecular testing may be required to objectively distinguish these two entities. Functionally, the EWSR1-ATF1 chimeric protein binds to and activates microphthalmia-associated transcription factor (MITF), a lineage-specific transcription factor important in melanocytic differentiation, possibly accounting for the shared histological and immunophenotypical features. While this gene fusion has also been described in several tumour types, including Ewing sarcoma, angiomatoid fibrous histiocytoma (AFH) and myoepithelioma of soft tissue, none of these have morphological overlap with CCS and melanoma.[11,12]

The most difficult differential diagnosis in our case was mucosal lentiginous melanoma (MLM), due to the intimate association with mucosal epithelium. Comparatively speaking, MLMs usually appear at an older age, are frequently heavily pigmented in the early stages, and most commonly involve the nasal mucosa. They do not arise from a pre-existing naevus. Histologically, they display lentiginous intraepithelial spread of spindled and dendritic melanocytes, and the proliferation usually expands radially beyond what is appreciated clinically. When they become invasive, MLM often show spindled cytology with loss of pigmentation. Although scarce, genetic studies of oral mucosal melanomas have reported activating mutations in *BRAF* and *CKIT*.[13]

The prognosis for our patient (survival rates of 67%, 33% and 10% at 5, 10 and 20 years, respectively) becomes worse over time compared to that seen in similarly staged melanoma,[14] and the treatment strategies are very different. Surgery is the mainstay of treatment for CCS, but melanoma is treated systemically with targeted or immunotherapy in both the adjuvant and metastatic settings. The current case raises awareness for clear cell sarcomas that can arise in the mucosa of the head and neck and mimic melanoma. In cases such as this, with limited sample and pseudo-intraepithelial nests, *EWSR1-ATF1* fusion detection by molecular techniques becomes paramount for proper diagnosis treatment.

Table 1 Clinical, morphological, immunohistochemical, and molecular features of oral CCS previously reported in the literature, including the current case

	Case no.						
	1	2	3	4	5	6	7
Site	Tongue	Parapharyngeal region	Tongue	Lip	Tongue	Jaw	Palate
Gender	F	F	M	M	M	F	M
Age	29	20	44	13	82	17	27
Size of the lesion, cm	NA	6	NA	NA	2	2.1	4
Microscopic architectural pattern	Nests and fascicles	Storiform-like with sheets	Sheets and nests	Plexiform	Sheets, nests, and fascicles of	NA	Sheets and nests
Cytology	Spindle-shaped cells	Epithelioid and spindle cells	Epithelioid and spindle cells	Epithelioid and spindle cells	Epithelioid and spindle cells	NA	Epithelioid and spindle cells
Extensive nests in the mucosa	−	−	−	−	−	NA	+
Junctional component	−	−	−	−	−	−	+
Pseudoalveolar pattern	−	−	−	−	+	NA	−
Multinucleated cells osteoclasts like	−	−	−	+	−	NA	−
Necrosis	−	−	−	−	−	NA	−
Mitotic count/ 10HPF	1	1	0	0	3	NA	12
Immunochemistry	S100+, HMB45+, MelanA−	MelanA+, HMB45+, S100 focally positive	S100+, HMB45+, MelanA−	S100+, HMB45 and MITF focally positive	S100+, HMB45+, MelanA−	S100+, HMB45+	MelanA+, S100+, HMB45+, Sox10+
EWSR1-ATF1 fusion	+	+	+	+	+	+	+
Treatment	Surgery	Surgery	NA	Surgery	XRT	Surgery	Surgery, XRT
Clinical evolution	NED	NA	NED	NED	NED	NED	NED
Follow-up, months	12	NA	NA	3	7	13	6
Reference	1	2	3	4	5	6	Present case

CT, chemotherapy; D, dermal; F, female; M, male; NA, not available; XRT, radiation therapy; NED, no evidence of disease.

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1. Singh M, Ieremia E, Debiec-Rychter M, *et al*. Clear cell sarcoma of the tongue. *Histopathology* 2014; 64: 750−1.
2. Fan C, Yu J, Yang L, *et al*. Clear cell sarcoma of soft tissue in right parapharyngeal region: report of a rare case. *Int J Clin Exp Pathol* 2015; 8: 10935−40.
3. Feasel PC, Cheah AL, Fritchie K, *et al*. Primary clear cell sarcoma of the head and neck: a case series with review of the literature. *J Cutan Pathol* 2016; 43: 838−46.
4. Sidiropoulos M, Busam K, Guitart J, *et al*. Superficial paramucosal clear cell sarcoma of the soft parts resembling melanoma in a 13-year-old boy: superficial paramucosal clear cell sarcoma. *J Cutan Pathol* 2013; 40: 265−8.
5. Kraft S, Antonescu CR, Rosenberg AE, *et al*. Primary clear cell sarcoma of the tongue. *Arch Pathol Lab Med* 2013; 137: 1680−3.
6. Inoue S, Chepeha DB, Lucas DR, *et al*. Clear cell sarcoma of the jaw: a case report and review of the literature. *J Pediatr Hematol Oncol* 2013; 35: 402−5.
7. Hantschke M, Mentzel T, Rütten A, *et al*. Cutaneous clear cell sarcoma: a clinicopathologic, immunohistochemical, and molecular analysis of 12 cases emphasizing its distinction from dermal melanoma. *Am J Surg Pathol* 2010; 34: 216−22.
8. Falconieri G, Bacchi CE, Luzar B. Cutaneous clear cell sarcoma: report of three cases of a potentially underestimated mimicker of spindle cell melanoma. *Am J Dermatopathol* 2012; 34: 619−25.
9. Zambrano E, Reyes-Mugica M, Franchi A, *et al*. An osteoclast-rich tumor of the gastrointestinal tract with features resembling clear cell sarcoma of soft parts: reports of 6 cases of a GIST simulator. *Int J Surg Pathol* 2003; 11: 75−81.
10. Libertini M, Thway K, Noujaim J, *et al*. Clear cell sarcoma-like tumor of the gastrointestinal tract: clinical outcome and pathologic features of a molecularly characterized tertiary center case series. *Anticancer Res* 2018; 38: 1479−83.
11. Gareton A, Pierron G, Mokhtari K, *et al*. ESWR1-CREM fusion in an intracranial myxoid angiomatoid fibrous histiocytoma-like tumor: a case report and literature review. *J Neuropathol Exp Neurol* 2018; 77: 537−41.
12. Cheah AL, Billings SD. The role of molecular testing in the diagnosis of cutaneous soft tissue tumors. *Semin Cutan Med Surg* 2012; 31: 221−33.
13. Chen F, Zhang Q, Wang Y, *et al*. KIT, NRAS, BRAF and FMNL2 mutations in oral mucosal melanoma and a systematic review of the literature. *Oncol Lett* 2018; 15: 9786−92.
14. Finley JW, Hanypsiak B, Mcgrath B, *et al*. Clear cell sarcoma: the Roswell Park experience. *J Surg Oncol* 2001; 77: 16−20.

DOI: https://doi.org/10.1016/j.pathol.2018.10.019