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Primary undifferentiated pleomorphic sarcoma in oral-maxillary area: retrospective study and molecular analysis

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Abstract

Undifferentiated pleomorphic sarcoma (UPS) in oral-maxillary area is rarely reported. Herein, we aimed to investigate the clinical characteristics, treatment strategies, prognosis, and molecular features of the oral-maxillary UPS. In total, 10 cases with primary oral-maxillary UPS were included. The rapidly progressive UPS can easily develop to an advanced and life-threatening stage, especially concerning the complex anatomical structures and spaces in the oral-maxillary area. The final diagnosis for UPS greatly depended on histological findings and immunohistochemistry staining after the exclusion of all possible differential diagnoses. Retrospectively, the treatment strategies for the included cases still referred to those of oral squamous cell carcinoma (OSCC). Statistically, the median overall survival (OS) for all the included cases was 7.75 months (range: 5-17 months). Comparatively, 3 cases had improved OS (median survival: 17 months, range: 17-18 months) and experienced PR/SD with neoadjuvant chemotherapy (anlotinib). The molecular features were demonstrated by using whole exonic sequencing for 1 included case. Cancer driver gene detection revealed GBP4 as a candidate driver gene for the primary oral-maxillary UPS. Additionally, a missense mutation in gene PIK3CA (p.E545K) was also identified. Our findings could greatly expand the knowledge about primary oral-maxillary UPS, and provide molecular evidences to improve the therapeutic options for primary oral-maxillary UPS.

Key words: Undifferentiated pleomorphic sarcoma; oral-maxillary area; retrospective study; whole exonic sequencing
Introduction

Undifferentiated pleomorphic sarcoma (UPS) has been established as an entity in the WHO-classification of soft tissue sarcoma (STS) since 2002 (Fletcher et al., 2002; Müller-Richter et al., 2008; Fletcher C, 2014). UPS is the fourth most common soft tissue sarcoma (STS), most of which occur in the extremities and the trunk of elderly patients (Srinivasamurthy et al., 2016; Moore et al., 2018). The etiology for UPS is unknown, and the clinical features of UPS are not specific (Widemann et al., 2018). Till now, the clinical characteristics and therapeutic experiences of primary UPS are limited, and patients suffering from UPS experience a poor prognosis (Yao et al., 2012; Jeong et al., 2016).

Accordingly, UPS in the head and neck area is relatively rare, with an incidence ranging from 4% to 10% (Patel et al., 2001; Bentz et al., 2004). The most frequently involved sites are neck and parotid glands, followed by scalp, face, anterior skull base and orbit (Patel et al., 2001; Bentz et al., 2004). Primary UPS involving the oral-maxillary area is rarely reported, and the clinical outcomes for patients with oral-maxillary UPS seem to be extremely poor. In this study, we managed to collect the primary oral-maxillary UPS treated in our department, retrospectively. The clinicopathological characteristics and treatment strategies for the included UPS patients were summarized and analyzed. Also, the molecular features were investigated by using whole exonic sequencing (WES) for 1 included case.
Patients and methods

Patients

Retrospectively, we reviewed and collected the UPS patients who were pathologically diagnosed and experienced multidisciplinary treatment between 2015 and 2020 at the Department of Oral Maxillofacial-Head and Neck Oncology, Shanghai Ninth People’s Hospital. To be included in this study, the following criteria were met for each case: (a) primary/recurrent UPS in the oral-maxillary area; (b) intact medical histories of diagnosis and treatments; (c) intact histopathological data; (d) intact imaging records; (e) follow-up information as complete as possible. The following cases were excluded: (a) the metastasized/secondary UPS involved the oral-maxillary areas; (b) patients with only histopathological biopsy and without subsequent treatments. This study was approved by the Medical Ethics Committee of the Ninth People’s Hospital, Shanghai Jiao Tong University School of Medicine (SH9H-2020-T321-1).

The demographic data of the involved patients, including sex, age, primary tumor site, clinical presentation, and previous radiation history, were summarized. The TNM staging for each involved case referred to the American Joint Committee on Cancer (AJCC) Cancer Staging manual for head and neck cancer (8th Edition, 2017). The clinical outcomes for each case were collected and summarized retrospectively from the follow-up information.
Imaging examinations

All patients received enhanced computed tomography (CT) and/or magnetic resonance imaging (MRI) examinations from the skull base to the clavicular level at their primary visit and during follow-up. Chest CT imaging was also performed for each case at the first visit and during follow-up. Representative CT/MRI images were downloaded from the Hospital Information System (HIS) of the hospital.

Microscopic observations

The final pathological reports were presented based on pathologic examinations by using haematoxylin-eosin (HE) and immunohistochemical (IHC) staining. For IHC staining, a routine panel for the diagnosis of malignant tumors was used. IHC staining was performed with primary antibodies against AE1/AE3 (pancytokeratin), CKH (Cytokeratin-high molecular weight), EMA (Epithelial membrane antigen), Vimentin, ki67, INI1 (Integrase interactor 1), CD31, Desmin, MyoD1, and ERG (v-ets erythroblastosis virus E26 oncogene homolog) (Autostainer/Autostainer Plus, DAKO, Denmark).

Whole Exonic Sequencing and Analysis

Genomic DNA was extracted via QIAamp DNA FFPE Tissue Kit (QIAGEN, Germany) from the entire paraffin embedded tissue of case 6. Gene Exonic regions were selected by Agilent SureSelect Human All Exon V6 Kit and library generated according to the manufacturer’s instructions (Agilent Technology, USA). DNA library
was sequenced on Illumina NovaSeq platform via PE150 protocol (Illumina, USA). About 30G Raw data (300x in depth) were trimmed and mapped to reference genome hg38 using Burrows-Wheeler Aligner (BWA, http://bio-bwa.sourceforge.net/) tool. PCR duplicates were removed using Picard (http://github.com/broadinstitute/picard).

Somatic SNP and Indel were discovered according to Genome Analysis Toolkit (GATK, http://software.broadinstitute.org/gatk/) workflow and annotated using ANNOVAR software with Refseq, CLINVAR, COSMIC, dbSNP150 database. Cancer driver gene detection was performed by maftools oncodrive function based on oncodrive CLUST algorithm (http://bitbucket.org/bbglab/oncodriveclust). Structure variants detection was performed by delly2 and annotated using ANNOVAR with DGV database (http://dgv.tcag.ca). Factera (https://factera.stanford.edu/) was used to detect the gene fusion in the genome. Control-Freee (http://boevalab.com/FREEC/) was used to detect the copy number variation. Microsatellite Instability (MSI) was detected by MSIsensor2 (https://github.com/niu-lab/msisensor2).

**Statistical analysis**

Statistical analyses were conducted using IBM SPSS Statistics version 20.0 software (IBM Corp., Armonk, NY, USA). The overall survival for the included cases was analyzed by Kaplan-Meier method. A p-value <0.05 was considered statistically significant.
Results

Clinical characteristics of the involved UPS patients

As shown in Table 1, of the 10 included UPS patients, 5 patients were male (median age at diagnosis 46.8 years, range 39-59 years), and 5 patients were female (median age at diagnosis 49.6 years, range 20-79 years). The primary sites included gingiva (4/10), tongue (3/10), buccal mucosa (1/10), palate (1/10), and maxillary sinus (1/10). Clinically, the most common symptom was the presentation of a rapidly and widely progressive soft-tissue mass. At the time of primary diagnosis, all patients developed tumors at the T3/T4 stage. In addition, no lymph node metastasis or distal metastasis was detected in any of the cases at their primary diagnosis. According to the medical histories of the included patients, 2 patients had records of OSCC history with previous radiation therapy.

Imaging characteristics of the UPS in oral-maxillary area

Imageological diagnosis based on CT and/or MRI was necessary pre-operation. During the preoperative radiological examination, enhanced CT demonstrated a solid mass with malignant characteristics (Figure 1). Based on the CT images of the included cases, we observed an extensive invasion of UPS to the hard/soft palate, nasal cavity, maxillary sinus, pterygopalatine fossa, skull base, orbit, parapharyngeal area, mandible, masticatory muscles, mouth floor, tongue base, oropharyngeal area, and carotid sheath (Figure 1A-E). As shown in Figure 1F-H, T2WI MRI and contrast-enhanced MRI confirmed a solid mass with heterogeneous signals and
heterogeneous enhancement. Besides, the CT/MRI did not indicate any lymph node metastasis. According to the aggressive clinical symptoms and CT/MRI findings, clinicians were able to recognize this soft-tissue mass as malignant tumor preoperatively.

**Histopathological characteristics of the UPS**

The final diagnosis for UPS greatly depended on histological findings and immunohistochemistry (IHC) staining (Table 2). The microscopic examination of HE-stained sections revealed a malignant neoplasm with numerous spindle-shaped fibroblasts intermingled in fascicles (Figure 2A). In high powered view, varying amounts of nuclear pleomorphism could be observed (Figure 2B). IHC analysis demonstrated a negative staining for the epithelial markers (AE1/AE3, CKH, EMA, Figure 2C-E), and a negative staining for Desmin and MyoD1 (Figure 2F, G). A positive staining for vimentin was observed for all the included cases (Figure 2H). Besides, high Ki-67 activity was widely observed (Figure 2I). Negative INI1 staining excluded the possibility of malignant peripheral nerve sheath tumor (Figure 2J). Notably, positive CD31 and ERG staining was observed in 5/10 patients (Figure 2K, L). Excluding all possible differential diagnoses and after discussion by Shanghai Clinical Pathology Reading Conference, the involved cases were eventually diagnosed as UPS.
**Treatment and prognosis of the primary UPS in oral-maxillary area**

Till now, the therapeutic strategies for UPS in oral-maxillary area have not reached a consensus. Retrospectively, the treatment strategies for the involved cases still referred to those of oral squamous cell carcinoma (OSCC) in our department. The treatment strategies and prognosis outcomes for all the included cases are summarized in Table 3. Five patients accepted neoadjuvant chemotherapy before operation, and 1 of them achieved partial response (PR) for anlotinib. A total of 10 patients underwent extensive resection of the mass (pathological confirmation for the resection margins as R0), and 2 of them combined with one-stage neck dissection. Soft-tissue reconstructions (ALTF/PMMF) were performed in 8 cases. Postoperatively, 6 patients were treated with adjuvant intensity-modulated radiotherapy (60-70 Gy beginning within 6 weeks postoperatively) and chemotherapy/target therapy. In addition, case 2 received adjuvant chemotherapy, and case 4 received adjuvant intensity-modulated radiotherapy only (60 Gy beginning at the fourth week postoperatively). Case 1 and case 6 did not accept adjuvant therapy postoperatively.

Generally, the outcomes for the UPS in oral-maxillary area were not encouraging. Statistically, the median survival for all the included cases was 7.75 months (range: 5-17 months) (Figure 3A). Three cases had improved OS (median survival: 17 months, range: 17-18 months) and experienced PR/SD with neoadjuvant chemotherapy (anlotinib) and adjuvant radiotherapy/chemotherapy compared with other cases (median survival: 6.25 months, range: 5-12 months; $p=0.0101$; Figure 3B). Seven cases died from the recurrence of the UPS in head and neck, and 2 cases died from
lung metastasis and the subsequent malignant pleural effusions (Figure 3C-D). In addition, 1 case died from recurrence and lung metastasis (Table 3).

**Molecular features of UPS in oral-maxillary area**

In this study, we described a rapidly progressive case of UPS originating from the buccal area (who responded poorly to neoadjuvant chemotherapy and died from lung metastasis, Figure 4A) and analyzed its mutation variations (Figure 4B). In total, we detected 739,096 SNVS (single nucleotide variants), 113,934 INDELs (insertion or deletion), 27, 514 SVs (structure variant), and 90 gene fusions (Figure 4B). Cancer driver genes detection revealed GBP4 as a candidate driver gene for the primary oral-maxillary UPS (Figure 4C). A total of 8 missense mutations were detected in exon 10 of the gene GPB4, including rs561042, rs561037, rs608339, rs1142890, rs1142889, rs1142888, rs1142886, and rs655260 (Figure 4D). We also identified a missense mutation in gene PIK3CA (rs104886003, Figure 4E). The WES data revealed a high TMB (tumor mutation burden, 152.01) in the detected case (Figure 4F). Comparatively, the MSI (microsatellite instability) rate of the case was detected as MSI-L/MSS (microsatellite instability-low/microsatellite instability-stable, Figure 4G).
Discussion

In this study, we managed to demonstrate the clinicopathological characteristics and molecular features of primary UPS in the oral-maxillary area for the first time. The rapidly progressive UPS could easily develop to an advanced and life-threatening stage (widespread invasion of soft tissues and bones), especially concerning the complex anatomical structures and spaces in the oral-maxillary area. Comparatively, regional lymph metastasis of UPS was rarely observed. The histopathological diagnosis for UPS greatly depended on the histological examination and extensive IHC markers to differentiate it from other malignant epithelial and mesenchymal tumors (Srinivasamurthy BC et al., 2016). The main differential diagnoses should be considered and excluded before the eventual diagnosis of UPS, including poorly differentiated carcinoma, melanoma, dedifferentiated liposarcoma, pleomorphic liposarcoma, pleomorphic leiomyosarcoma, myxofibrosarcoma, pleomorphic rhabdomyosarcoma, and malignant peripheral nerve sheath tumor (Patel et al., 2001; Bentz et al., 2004; De Vita et al., 2016; Widemann et al., 2018). Previous studies have demonstrated that UPS is the most common histologic subtype of radiation-associated sarcoma (RAS) (Koyama et al., 2014; Dineen et al., 2015), and 2/10 of the included UPS patients had previous radiation-therapy history. So, radiation-induced UPS could not be ignored for patients with radiation history in the oral-maxillary area.

Accordingly, surgical resection with negative resection margins is still the mainstay of treatment for UPS (Patel et al., 2001; Bentz et al., 2004; Yao et al., 2012). In our department, radical resection with negative margins had been performed for the
UPS patients. Neck dissection was not regularly recommended for the rare cervical metastasis of UPS patients. When comparing the resected range with the UPS originating from other sites, the wide tissue-defects in the oral-maxillary area could greatly affect the quality of life for the patients. In our department, simultaneous soft-tissue flaps were recommended to reconstruct the composite tissue defects of the oral-maxillary system. Besides, treatment strategies for the oral-maxillary UPS have been developed through collaboration among clinicians within a multidisciplinary team (MDT) in our department. As recurrence was common and 5-year OS was poor for the UPS patients, radiation therapy and (neo)adjuvant chemotherapy were regularly recommended to reduce local and distant recurrence. Herein, considering the poor prognosis of oral-maxillary UPS, MDT treatment strategy was recommended including neoadjuvant/targeted chemotherapy, radical surgery, adjuvant chemotherapy/targeted therapy, and adjuvant radiation. Comparatively, the prognosis of UPS arising from oral-maxillary area was much poorer when compared with UPS in other regions (Sabesan et al., 2006; Zhu et al., 2018).

Chemotherapy is recommended for the treatment of these rapidly progressive tumors, but conventional chemotherapy offers limited efficacy, and is often poorly tolerated and associated with significant toxicity (De Vita et al., 2017; Keung et al., 2018). Hence, there is an urgent need to develop new therapies for oral-maxillary UPS. It has been generally acknowledged that angiogenesis plays crucial roles in malignant aggressiveness in a variety of solid tumors (Miyata et al., 2015; Zhang et al., 2018; Mohamed et al., 2019). Microvascular density (MVD) is still measured to
evaluate the histopathological angiogenesis in tumors (Miyata et al., 2015). The panvascular endothelial marker CD31 has been widely termed as a relatively sensitive and specific indicator for MVD in tumors (Mohamed et al., 2019). MVD assessed by CD31 has been reported to predict clinical benefit upon bevacizumab (an anti-vascular endothelial growth factor monoclonal antibody) (Bianconi et al., 2020). In this study, positive CD31 staining was observed in 5/10 patients. Comparatively, better responses to the neoadjuvant chemotherapy (anlotinib) were observed for the 3 cases with CD31 positive staining. Anlotinib is a novel, orally administered, multi-target receptor tyrosine kinase inhibitor, which functions by inhibiting tumor angiogenesis and proliferative signaling pathways (Sun et al., 2016). Till now, a series of preclinical and clinical studies have shown that anlotinib is effective and safe in the treatment of multiple solid tumors (Wang et al., 2020). High-dose anlotinib combined with epirubicin has been reported to be an effective and safe therapy for STS (Wang et al., 2020). Herein, the efficacy of anlotinib was primarily summarized and proposed in the treatment of oral-maxillary UPS, and well-designed prospective clinical trials should be done to confirm our findings.

Recently, the molecular characterization of UPS originating from other tissues has been demonstrated sporadically (Li et al., 2015; Lewin et al., 2018). Activation of Hedgehog and Notch signaling pathways have been demonstrated in cells with tumor-initiating potential from UPS (Widemann et al., 2018; Amm et al., 2020). Besides, VGLL3 and YAP1 in the Hippo signaling pathway have been shown to be overexpressed in a subset of UPS (Widemann et al., 2018). However, studies on the
UPS mutational landscape are rarely conducted. This study was the first to use the WES to detect the gene mutation occurring in the primary oral-maxillary UPS. In the detected UPS case, cancer driver gene detection revealed GBP4 as a candidate driver gene. The GBP4s are the members of the guanosine triphosphatase family (GTPases) that are induced by IFNγ and have a role in resistance to pathogens (Tyrkalska et al., 2016). A high expression of GBP1, GBP2, GBP3, GBP4 and GBP5 have been observed to be correlated to more favorable clinical outcomes in colorectal cancer, skin cutaneous melanoma, breast cancer, OSCC, esophageal squamous cell carcinoma, and Kaposi’s sarcoma (Britzen-Laurent et al., 2013; Wang et al., 2018). In this study, GBP4 was bioinformatically predicted as a candidate driver gene, indicating that the mutations of GBP4 might play key roles in the oncogenesis of oral-maxillary UPS. Nonetheless, more studies are still needed to investigate the roles of mutated GBP4 in the oncogenesis of UPS in detail. Additionally, a missense mutation in gene PIK3CA (p.E545K) was also identified. Till now, the mutational activation of the PI3K pathway has been demonstrated as a central event in many types of cancer (Beaver et al., 2013). A high frequency of mutations in the PIK3CA gene occur in the “hotspots” located in exon 9 (E542K, E545K) and exon 20 (H1047R), which are oncogenic in a wide variety of human cancers (Kang et al., 2005). Accordingly, PIK3CA E545K cells showed increased proliferation rates in all growth conditions compared with wild-type cells (Beaver et al., 2013). So, the mutation of PIK3CA might greatly contribute to the malignant progression of the primary oral-maxillary UPS, which should be further validated in the future. The WES data revealed a high TMB (tumor mutation burden,
in the detected case, which has emerged as a promising predictive biomarker for immune checkpoint inhibitor therapy (Alborelli et al., 2020).

In this study, a retrospective analysis for oral-maxillary UPS was conducted and shared. A main limitation of this study was the limited sample size from a single clinical center. Besides, great heterogeneity existed among the neoadjuvant/adjuvant chemotherapies for the included cases. The detailed chemotherapy regimens could not been analyzed and discussed. For molecular analysis, our study was limited by the inclusion of one patient for WES analysis, and also limited by the lack of further molecular and biological validation.

In conclusion, our findings could greatly expand the knowledge about the mutation spectrum and oncogenesis of oral-maxillary UPS, and provide molecular evidence to improve the therapeutic options for UPS patients. Further studies are warranted to validate our discoveries in the oral-maxillary UPS.

Acknowledgements

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Competing interests

All the authors declare that they have no competing interests.
**References**


adipocytic sarcomas. Onco. Targets Ther. 9, 6233-6246.


Figure legends

Figure 1: Representative CT/MRI images showing the local invasion of UPS in oral-maxillary area. (A-E) CT images: (A) Invasion of the hard/soft palate, nasal cavity, maxillary sinus, and skin (Case 9); (B) Invasion of the maxilla and pterygopalatine fossa (Case 4); (C) Invasion of the skull base and orbit (Case 10); (D) Invasion of the parapharyngeal area, mandible, masticatory muscles, and mouth floor (Case 3); (E) Invasion of the tongue base, mouth floor, oropharyngeal area, and carotid sheath (Case 7). (F-H) MRI images: (F) T1WI (Case 9); (G) Contrast-T1WI (Case 9); (H) T2WI (Case 9).

Figure 2: Representative histopathological images for the UPS in oral-maxillary area. (A-B) H&E stained sections: (A) 100x; (B) 200x. (C-L) Immunohistopathological characteristics: (C) AE1/AE3; (D) CKH; (E) EMA; (F) Desmin; (G) MyoD1; (H) Vimentin; (I) Ki67; (J) INI1; (K) CD31; (L) ERG.

Figure 3: Prognosis analysis of the UPS cases in oral-maxillary area. (A) Analysis of overall survival for all the involved cases; (B) Analysis of overall survival stratified by the response of neoadjuvant chemotherapy; (C) Pulmonary nodules indicating lung metastasis (red arrow, Case 10); (D) Malignant pleural effusions resulted from lung metastasis of the UPS (red arrow, Case 6).
Figure 4: Medical history and molecular analysis for case 6. (A) Medical history for case 6: treatment strategies and disease progression were indicated at each time point by black arrows; (B) Circos plot summarized all the genetic mutations detected by WES analysis; (C) GBP4 was identified as a candidate cancer driver gene; (D) Location of the detected mutation sites for GBP4; (E) Location of the detected mutation site for PIK3CA; (F) Tumor mutation burden analysis; (G) Microsatellite instability analysis.
Table 1 Clinical features of 10 oral-maxillary UPS cases.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/Sex</th>
<th>Primary Site</th>
<th>Invasion Range</th>
<th>TNM stage</th>
<th>Previous radiation</th>
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<td>1</td>
<td>45/M</td>
<td>R upper gingiva</td>
<td>R buccal mucosa; R palate; R maxillary sinus</td>
<td>T4N0M0</td>
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<tr>
<td>2</td>
<td>42/M</td>
<td>L tongue</td>
<td>L mouth floor</td>
<td>T3N0M0</td>
<td>Yes</td>
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<tr>
<td>3</td>
<td>46/F</td>
<td>R lower gingiva</td>
<td>R masticatory muscles; R mouth floor; R parapharyngeal area</td>
<td>T4N0M0</td>
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<td>4</td>
<td>63/F</td>
<td>L upper gingiva</td>
<td>L buccal mucosa; L pterygomandibular fold; L maxillary sinus</td>
<td>T4N0M0</td>
<td>No</td>
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<tr>
<td>5</td>
<td>39/M</td>
<td>L tongue</td>
<td>L mouth floor; L medial pterygoid; L parapharyngeal area</td>
<td>T4N0M0</td>
<td>No</td>
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<tr>
<td>6</td>
<td>79/F</td>
<td>L buccal area</td>
<td>L maxilla; L Lip; L buccal mucosa; skin</td>
<td>T4N0M0</td>
<td>No</td>
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<tr>
<td>7</td>
<td>49/M</td>
<td>R tongue</td>
<td>R mouth floor;</td>
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<td>8</td>
<td>20/F</td>
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<td>9</td>
<td>59/M</td>
<td>L upper gingiva</td>
<td>L maxilla; L pterygopalatine fossa; Skin</td>
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<tr>
<td>10</td>
<td>40/F</td>
<td>R maxillary sinus</td>
<td>R orbit; R pterygopalatine fossa;</td>
<td>T4N0M0</td>
<td>No</td>
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</table>

Note: M: male; F: female; R: right; L: left.
Table 2 Immunohistopathological characteristics for the cases with primary oral-maxillary UPS.

<table>
<thead>
<tr>
<th>Case</th>
<th>AE1/AE3</th>
<th>CKH</th>
<th>EMA</th>
<th>Desmin</th>
<th>MyoD1</th>
<th>Vimentin</th>
<th>Ki67</th>
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Note: -: negative staining; +: positive staining.
Table 3: Treatment strategies and clinical outcomes for the 10 cases with oral-maxillary UPS.

<table>
<thead>
<tr>
<th>Case</th>
<th>Neoadjuvant chemotherapy</th>
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<th>Radiation</th>
<th>Chemotherapy/Target therapy</th>
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<td>No</td>
<td>Extensive Resection</td>
<td>No</td>
<td>No</td>
<td>Died at 9 m (Recurrence)</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>Extensive Resection + SOND + ALTF</td>
<td>No</td>
<td>Yes</td>
<td>Died at 5 m (Recurrence)</td>
</tr>
<tr>
<td>3</td>
<td>Anlotinib (PR)</td>
<td>Extensive Resection + ALTF</td>
<td>Yes</td>
<td>Yes</td>
<td>Died at 18 m (Metastasis)</td>
</tr>
<tr>
<td>4</td>
<td>No</td>
<td>Extensive Resection</td>
<td>Yes</td>
<td>No</td>
<td>Died at 5 m (Recurrence)</td>
</tr>
<tr>
<td>5</td>
<td>UN (PD)</td>
<td>Extensive Resection + RN+ PMMF</td>
<td>Yes</td>
<td>Yes</td>
<td>Died at 5 m (Recurrence)</td>
</tr>
<tr>
<td>6</td>
<td>MAID (PD)</td>
<td>Extensive Resection + ALTF</td>
<td>No</td>
<td>No</td>
<td>Died at 6.5 m (Metastasis)</td>
</tr>
<tr>
<td>7</td>
<td>No</td>
<td>Extensive Resection + ALTF</td>
<td>Yes</td>
<td>Yes</td>
<td>Died at 6 m (Recurrence)</td>
</tr>
<tr>
<td>8</td>
<td>Anlotinib (SD)</td>
<td>Extensive Resection + ALTF</td>
<td>Yes</td>
<td>Yes</td>
<td>Died at 17 m (Recurrence)</td>
</tr>
<tr>
<td>9</td>
<td>No</td>
<td>Extensive Resection + ALTF</td>
<td>Yes</td>
<td>Yes</td>
<td>Died at 12 m (Recurrence)</td>
</tr>
<tr>
<td>10</td>
<td>Anlotinib (SD)</td>
<td>Extensive Resection + ALTF</td>
<td>Yes</td>
<td>Yes</td>
<td>Died at 17 m (Recurrence + Metastasis)</td>
</tr>
</tbody>
</table>

Note: PR: partial response; PD: progressive disease; SD: stable disease; UN: unknown; SOND: supraomohyoid neck dissection; RD: radical neck dissection; ALTF: Anterolateral thigh flap; PMMF: Pectoralis major myocutaneous flap.
A

Median survival: 7.75 m
Range: 5 m-17 m

B

PR/SD cases with neoadjuvant chemotherapy
Median survival: 17 m
Range: 17 m-18 m

Other cases
Median survival: 6.25 m
Range: 5 m-12 m

p = 0.0101
A

- Neoadjuvant chemotherapy with MAID
- Rapidly growing
- Treated with traditional Chinese medicine
- Died from malignant pleural effusion
- Lung metastasis
- Radical Surgery and Pathological Diagnosis

B

- Missense mutations
- Exon10:c.G1635C:p.M545I (rs1142889)
- Exon10:c.A1633C:p.M545L (rs1142888)

C

- Fraction of variants within clusters
- Amino acid sequence (GBP4)

D

- Tumor Mutation Burden: 152.01
- Status: HIGH
- Total Scan Sites: 2079
- Detected Somatic Sites: 35
- Rate (%): 1.68 & MSI-L/MSS