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Research Paper
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A pilot study for presence of circulating tumour cells in adenoid cystic carcinoma

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Abstract. Adenoid cystic carcinoma (ACC) is a rare salivary gland neoplasm with a poor long-term prognosis due to multiple recurrences and distant metastatic spread. Circulating tumour cells (CTCs) are tumour cells shed from a primary, recurrent, or metastatic cancer that are detectable in the blood or lymphatics. There is no literature to date confirming the presence of CTCs in ACC. The aim of this study was to determine whether CTCs are detectable in ACC. Blood samples were collected from eight patients with histologically confirmed ACC. The TNM stage of the tumour was recorded, as well as any prior treatment. CTCs were isolated by spiral microfluidics and detected by immunofluorescence staining. Three of the eight patients recruited (32.5%) had staining consistent with the presence of CTCs. Of these three patients with detectable CTCs, one had confirmed pulmonary metastasis, one had suspected pulmonary metastasis and was awaiting confirmation, and one had local recurrence confirmed on re-resection. One patient with known isolated pulmonary metastasis had previously undergone a lung metastasectomy and did not have CTCs detected. CTCs are detectable in ACC. In this small patient sample, CTCs were found to be present in those patients with recurrent local disease and known distant metastatic disease. CTCs in ACC should be investigated further for their potential use as an adjunct in staging, prognosis, and the detection of recurrence.

Keywords: Carcinoma; Adenoid cystic; Neoplastic cells; Circulating; Salivary gland neoplasms; Neoplasm recurrence; Microfluidics; Flourescent antibody technique.

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Introduction

Adenoid cystic carcinoma (ACC) is a rare malignant tumour of salivary gland origin with an incidence of 3–4.5 cases per 1,000,000 people[1,2]. It accounts for 1% of all head and neck malignancies and 10–27.9% of all salivary gland tumours, and is one of the most common cancers of the major salivary glands[3–5]. ACC occurs in all age groups, with a higher frequency in middle-aged patients (fifth and sixth decades of life), and has no identifiable risk factors[6,7]. This carcinoma is characterized by perineural invasion and multiple recurrences. The prognosis is generally poor and therefore many clinicians consider ACC to be a 'clinically high-grade' neoplasm[3].

The treatment of ACC traditionally includes radical surgical resection

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whenever possible. This is typically followed by postoperative radiation therapy in the majority of cases. Regional lymph node metastasis is infrequent, reported to be found in 18.6% of all cases, but this may be a factor of infrequently performed neck dissections and lack of detailed assessment of retrieved lymph nodes[8]. Haematogenous metastasis to lung, bone, and liver is common[9,10]. Chemotherapy has been studied, but due to the indolent natural course of the disease, it has been difficult to observe the clinical response. Reported 5-year survival rates as high as 92% may give false optimism[11]. Long-term overall survival rates at 10 and 20 years are 52–65% and 28–40%, respectively[12–14].

Circulating tumour cells (CTCs) are described as tumour cells detectable in the blood or lymphatics, and CTCs have been described in patients with head and neck cancers, among other cancer types[15–17]. These cells are shed from primary, recurrent, or metastatic cancer. CTCs must undergo a complex transitional process known as epithelial to mesenchymal transition (EMT), which is the gateway to blood intravasation[18]. CTCs can lose their epithelial antigens, gain migratory and invasive properties causing disruption of the basement membrane, and present with a mesenchymal-like phenotype[19]. Once in the blood, platelets provide a microenvironment, forming a 'platelet cloak' that may shield CTCs from immune detection[20].

The presence of CTCs in peripheral blood is extremely rare, with detection in the order of 1 per 10^8–10^9 blood cells[21]. The half-life of CTCs is as short as 2 hours in some solid tumours, as they are often destroyed by immunological processes, blood shear forces, or apoptosis[22,23]. At the secondary site, CTCs have to change their new microenvironment in order for it to provide sufficient nutrients and oxygen, before they can redevelop as macrometastases. The reverse process of EMT, mesenchymal to epithelial transition (MET), is believed to play a role in allowing CTCs to 'seed' distant organs such as the lungs, liver, and bone, forming metastases[18,24]. CTCs are therefore recognized as a key intermediate step in the development of tumour metastasis.

CTCs were first discovered in 1869, but it was not until 2005 that the clinical significance of the presence of these cells was proven. It was found that the presence of these cells was associated with a poor prognosis[25]. The existence of CTCs in the bloodstream has been identified as a promising diagnostic, prognostic, and predictive biomarker (including for disease monitoring and the response to therapy) for a variety of human cancer types, including head and neck, lung, and breast cancers. In the staging of breast cancer, the presence of CTCs in cM0 disease is associated with an adverse prognosis for recurrence and survival. For those patients staged M1, a higher number of CTCs at the time of diagnosis has been strongly correlated with decreased survival[26].

Investigations have been performed to determine the presence of CTCs in head and neck squamous cell carcinoma (HNSCC) patients, with CTCs being detected in 12.5–77% of these patients[15,27]. This wide range is due to study heterogeneity, differing methods of detection, and the inclusion of all tumour stages. A decrease in CTCs within the first month following chemo-radiation treatment for HNSCC has been found to be associated with longer progression-free survival and longer overall survival[28].

ACC has been shown to have a relatively long tumour doubling time, on average 393 days, which may account for the poor long-term prognosis due to lung metastasis at >5 years after initial diagnosis. In fact, the onset of pulmonary metastasis has been hypothesized to be much earlier (227 months) than the initial visit[29]. Given this early propensity for early metastasis, the presence of CTCs may heighten the search for occult distant disease. This may allow improved staging, or the detection of recurrence. It appears that no study to date has investigated the presence of CTCs in ACC. The purpose of this study was to investigate the possibility of detecting CTCs in ACC patients. A secondary aim was to determine the association between CTC detection rates and the clinical outcome in ACC patients, as this may offer an opportunity for the development of a precision medicine approach for these patients.

Materials and methods

Study design

This study was approved by the Ethics Committee of the Princess Alexandra Hospital, Brisbane, Queensland (HREC Number HREC/12/QPAH/381). A total of eight patients who had been diagnosed with ACC of the head and neck were recruited from the Oral and Maxillofacial Surgery Department, the Royal Brisbane and Women's Hospital between October 2018 and May 2019. For all of these patients, the clinical stage of ACC was classified according to the American Joint Committee on Cancer/the Union for International Cancer Control (AJCC/UICC) tumour–node–metastasis (TNM) staging system (eighth edition of the AJCC Cancer Staging Manual)[26]. All participants gave written informed consent prior to sampling.

CTC enrichment by spiral microfluidics

CTCs were isolated from the blood samples of ACC patients using a spiral microfluidic-based technology, as described previously[27,30]. Briefly, 10-ml whole blood samples were collected in ethylenediaminetetraacetic acid (EDTA) blood collection tubes and transported immediately to the laboratory for downstream processing. An initial red blood cell lysis (Astral Scientific Pty Ltd, Taren Point, NSW, Australia) was performed to minimize the cellular components passing through the spiral chip. The pellet was then resuspended in 10 ml of sheath buffer (1 × phosphate buffered saline (PBS), 2 mM EDTA, 0.5% bovine serum albumin (BSA)), before loading into a 10-ml syringe (Thermo Fisher Scientific, Rockford, IL, USA). Next, the samples were pumped through the spiral chip at a flow rate of 1.7 ml/min. Finally, both enriched CTC (CTC^{Hi}) and depleted CTC/white blood cells (WBCs) (CTC^{Low}) were collected and fixed with 4% paraformaldehyde in PBS. After 10 minutes of fixation, both CTC^{Hi} and CTC^{Low} were spun onto slides using a Cytospin centrifuge (Thermo Fisher Scientific) at 1200 rpm for 5 minutes. All of the glass slides were stored at −80 °C for subsequent analysis.

CTC characterization using immunofluorescence staining

Immunofluorescence staining of specific antigens for cancer cells and WBCs was performed for CTC characterization. Briefly, the glass slides were blocked with 2% BSA (Sigma-Aldrich, St. Louis, MO, USA) in PBS for 1 hour at room temperature. The glass slides were then washed three times with PBS and incubated with human pan-cytokeratin (CK) eFluor 570-conjugated antibody (Thermo Fisher Scientific) and human CD45 allophycocyanin (APC)-conjugated antibody (BD Biosciences, San Jose, CA, USA), as well as their respective isotype control antibodies. After 1 hour of incubation, the glass slides were washed three times with PBS and subsequently incubated with 4′,6-diamidino-2-phenylindole (DAPI) for 5 minutes at room temperature before mounting with ProLong Antifade Reagents (Thermo Fisher Scientific). The fluorescence was determined using a Zeiss Axio Imager

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Z2 microscope (Zeiss, Oberkochen, Germany). CTCs were identified by the following criteria: CK-positive (green), CD45-negative (red), DAPI-positive (blue), a high nuclear–cytoplasmic ratio, and size larger than leukocytes. (Fig. 1)

Results

Eight patients with confirmed ACC had blood samples taken for analysis. The characteristics of these eight patients with histopathologically confirmed ACC are shown in Table 1. Four of the patients were female and four were male. The mean age at sample collection was 55.9 years (range 46–67 years). Six of the patients (75%) had an original tumour stage of T4. The most common primary site was the maxilla and/or palate (75%). Only one of the patients (12.5%) had evidence of regional metastasis on clinical imaging or final histopathology. Six of the patients (75%) had previously undergone surgical resection of the primary tumour, and in all instances this was followed by postoperative radiation therapy on recommendation of the hospital head and neck multidisciplinary team. Two of the patients (25%) had known metastatic disease, with biopsy-proven pulmonary metastatic deposits present. One of these patients had undergone a lung metastasectomy for the treatment of an isolated metastasis.

Three of eight patient samples (37.5%) stained positive for CK, negative for CD45, and positive for DAPI, consistent with the presence of CTCs. Of the three patients with detectable CTCs, one had confirmed pulmonary metastasis, one had suspected pulmonary metastasis and was awaiting confirmation, and one had local recurrence confirmed on re-resection. Five patients (62.5%) did not have CTCs detected with these staining methods. One patient with known isolated pulmonary metastasis had previously undergone a lung metastasectomy and did not have CTCs detected.

Discussion

ACC is a rare salivary gland neoplasm with a poor long-term prognosis due to multiple recurrences and distant metastatic spread[1–3]. In this pilot study, it was found that CTC detection was associated with the clinical disease status in ACC patients.

Based on the results of the staining, three of the eight patients (37.5%) were found to have CTCs. Patient 3, known to have untreated primary disease and two lung metastases on imaging, was found to have CTCs. These results could be expected given his clinical staging. Patient 6 was 5 years from his original surgical and postoperative radiation therapy. He has subsequently been diagnosed with mesothelioma. Interestingly, his latest chest imaging showed the presence of nodules not typical of mesothelioma, and he is set to have further investigations as this may represent metastatic ACC. Patient 7 had undergone two prior resections of the maxilla and nasal septum for the treatment of recurrent ACC, both followed by postoperative radiation therapy. She subsequently developed Notani grade 3 osteoradionecrosis of her entire remaining maxilla, necessitating a maxillectomy and composite free flap reconstruction. On histopathological analysis of the maxillectomy specimen, small foci of ACC were identified. The presence of CTCs in the setting of multiply locally recurrent ACC is in keeping with the aggressive nature of this particular tumour.

Of the five patients who did not have detectable CTCs, none had clinical evidence of local recurrence. Interestingly, patient 5 had undergone a lung metastasectomy for an isolated ACC metastasis only 1.5 months prior to sample collection. His sample did not demonstrate CTCs. This may demonstrate that metastasectomy is a reasonable option for isolated pulmonary metastasis, as has been argued previously[31]. The clinical value of CTCs for those undergoing curative intent pulmonary metastasectomy for colorectal cancer has been explored; it was

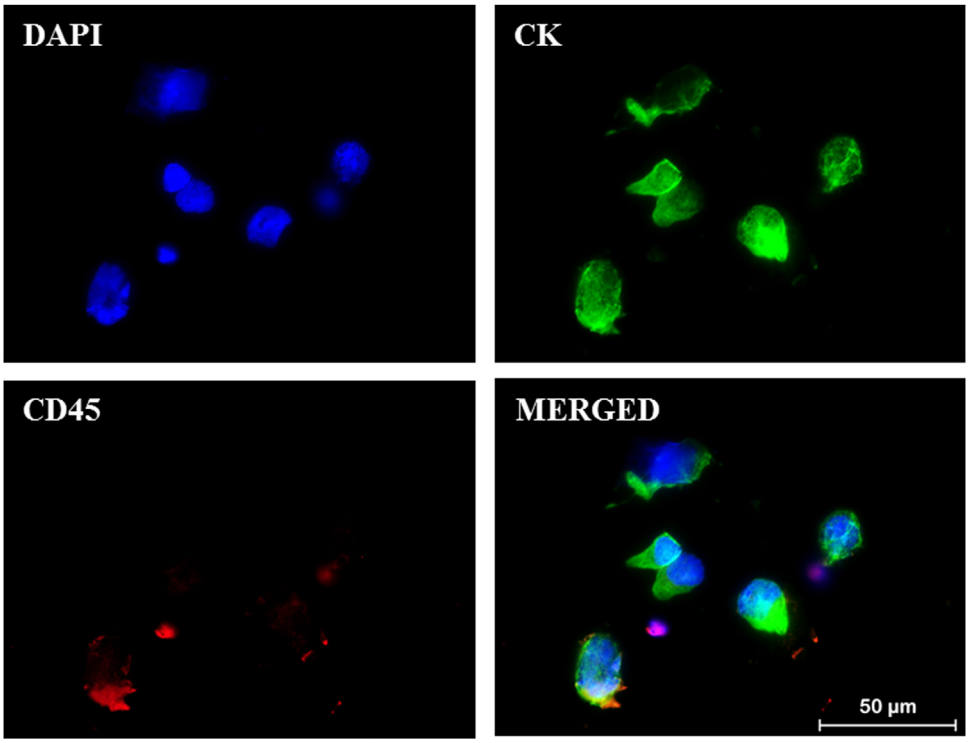

Fig. 1. Detection of CTCs in adenoid cystic carcinoma patients. Representative flourescent images of CTCs identified by the spiral microfluidic-based technology in combination with immunofluorescence staining on specific entigen for nuclear (DAPI, Blue), CTCS (CK, Green) and WBCs (CD45, Red). CTCs define as DAPI positive, CD45 negative and CK positive.

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Table 1. Demographic and clinical information of the patient population.

Patient	Age (years)	Sex	TNM[a]	Primary anatomical site	Previous local treatment	Local recurrence	Metastatic treatment	Active known disease	Presence of CTCs[b]
1	67	F	pT4aN0M0	Nasal floor	Surgery + PORT Feb 2018	No	N/A	No	No
2	58	F	pT4aN1M0	Maxilla	Surgery + PORT Sept 2017	No	N/A	No	No
3	47	M	cT3N0M1 (Lung nodules ×2)	Sublingual gland	None	N/A	No	Yes (local and pulmonary)	Yes
4	46	M	pT4aN0M0	Palate	Surgery + PORT Dec 2018	No	N/A	No	No
5	48	M	pT2N0M1 (Lung nodule)	Palate	Surgery + PORT Dec 2013	No	Yes (lung metastectomy Dec 2018, prior to sample collection)	No	No
6	67	M	pT4aN0M0	Maxilla	Surgery + PORT Sept 2014	No	N/A	Yes (suspected pulmonary)	Yes
7	54	F	pT4aN0M0	Palate	Surgery + PORT 2009, 2016, 2019	Yes ×2	N/A	Yes (local)	Yes
8	60	F	cT4bN0M0	Palate into base of skull	None	No	N/A	No	No

CTCs, circulating tumour cells; F, female; M, male; N/A, not applicable; PORT, postoperative radiation therapy.
[a] TNM staging as per the eighth edition of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual[26].
[b] Presence of CTCs defined as staining CK-positive, CD45-negative, and DAPI-positive.

demonstrated that those with multiple CTCs had a significantly shorter disease-free survival and overall survival[32].

The detection of CTCs in ACC in this small sample occurred in those patients with recurrent local disease and distant metastatic disease. The significance of the presence of CTCs is unknown for different cancer types (e.g. breast cancer vs ACC) and equating significance for ACC is beyond the scope of this pilot study. Future studies with larger sample sizes, adequate power, and longer term follow-up are required to answer that question. CTCs may potentially be useful as an adjunct in the staging of ACC or the detection of recurrence, as they are with some other cancers (e.g. breast cancer)[25]. This could improve initial treatment discussions with patients, with increased accuracy of staging. As adjunctive modalities such as immunotherapy continue to be explored, they may one day be used for the prevention or treatment of micrometastases. The detection of CTCs would be a key step in this type of tailored management. It could also prompt further imaging should CTCs be detected on follow-up. Further studies to determine a threshold number of CTCs in ACC should be undertaken, along with a larger series to validate the presence of CTCs with known local, regional, or metastatic disease, which may improve their clinical utility when they are detected.

In conclusion, this study demonstrated that CTCs can be detected in ACC; they were identified in 37.5% of patients in this study, confirmed by immunostaining. In this small patient sample, CTCs were found to be present in those patients with recurrent local disease and known distant metastatic disease. No CTCs were found in patients without radiological or clinical evidence of disease. The presence of CTCs in ACC should be investigated further, as they may have the potential to be used as an adjunct in staging, prognosis, and the detection of recurrence.

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Competing interests

The authors have no conflicts of interest to declare.

Ethical approval

HREC Reference Number HREC/12/QPAH/381.

Patient consent

Written patient consent was obtained prior to specimen collection.

References

1. Bjørndal K, Krogdahl A, Hamilton M, Overgaard J, Johansen J, Kristensen CA, Homoe P, Soreson CH, Anderson E, Bundgaard T, Primdahl H, Lambertsen K, Andersen LJ, Godballe C. Salivary gland carcinoma in Denmark 1990–2005: a national study of incidence, site and histology. Results of the Danish Head and Neck Cancer Group (DAHANCA). *Oral Oncol* 2011;**47**:677–82. http://dx.doi.org/10.1016/j.oraloncology.2011.04.020.
2. Bonaparte J, Hart R, Trites J, Taylor M. Incidence of adenoid cystic carcinoma in Nova Scotia: 30-year population-based epidemiologic study. *J Otolaryngol Head Neck Surg* 2008;**37**:642–8.
3. Coca-Pelaz A, Rodrigo JP, Bradley PJ, Vander Poorten V, Rinaldo A, Haigentz M, Takes RP, Mondin V, Teymorrtash A, Thompson LDR, Ferlito A. Adenoid cystic carcinoma of the head and neck—an update. *Oral Oncol* 2015;**51**:652–61. http://dx.doi.org/10.1016/j.oraloncology.2015.04.005.
4. Dodd RL, Slevin NJ. Salivary gland adenoid cystic carcinoma: a review of chemotherapy and molecular therapies. *Oral Oncol* 2006;**42**:759–69.
5. Vander Poorten VL, Balm AJ, Hilgers FJ, Tan IB, Loftus-Coll BM, Keus RB, Hart AA. Prognostic factors for long term results of the treatment of patients with malignant submandibular gland tumors. *Cancer* 1999;**85**:2255–64.
6. Nascimento A, Amaral A, Prado L, Kligerman J, Silveira T. Adenoid cystic carcinoma of salivary glands. A study of 61 cases with clinicopathologic correlation. *Cancer* 1986;**57**:312–9.
7. Zyrko E, Golubovic M. Laryngeal adenoid cystic carcinoma. *Acta Otorhinolaryngol Ital* 2009;**29**:279–82.
8. Ferlito A. Cervical lymph node metastasis in adenoid cystic carcinoma of the major salivary glands. *J Laryngol Otol* 2017;**131**:96–105. http://dx.doi.org/10.1017/S0022215116009749.
9. Stell PM. Adenoid cystic carcinoma. *Clin Otolaryngol Allied Sci* 1986;**11**:267–91.
10. Kokemuller H, Eckardt A, Brachvogel P, Hausamen J. Adenoid cystic carincoma of the head and neck—a 20 years experience. *Int J Oral Maxillofac Surg* 2004;**33**:25–31.
11. DeAngelis A, Tsui A, Wiesenfeld D, Chandu A. Outcomes of patients with adenoid cystic carcinoma of the minor salivary glands. *Int J Oral Maxillofac Surg* 2011;**40**:710–4.
12. van Weert S, Bloemena E, van der Waal I, de Bree R, Rietveld DH, Kuik JD, Leemans CR. Adenoid cystic carcinoma of the head and neck: a single center analysis of 105 consecutive cases over a 30-year period. *Oral Oncol* 2013;**49**:824–9.
13. Ciccolallo L, Licitra L, Cantu G, Gatta G, EUROCARE Working Group. Survival from salivary glands adenoid cystic carcinoma in European populations. *Oral Oncol* 2009;**45**:669–74.
14. Jones A, Hamilton J, Rowley H, Husband D, Helliwell T. Adenoid cystic carcinoma of the head and neck. *Clin Otolaryngol Allied Sci* 1997;**22**:434–43.
15. Kulasinghe A, Perry C, Jovanovic L, Nelson C, Punyadeera C. Circulating tumour cells in metastatic head and neck cancers. *Int J Cancer* 2015;**136**:2515–23. http://dx.doi.org/10.1002/ijc.29108.
16. Kulasinghe A, Perry C, Warkiani ME, Blick T, Byrne KO, Thompson EW, Nelson CC, Vela I, Punyadeera C. Short term ex-vivo expansion of circulating head and neck tumour cells. *Oncotarget* 2017;**7**:60101–9. http://dx.doi.org/10.18632/oncotarget.11159.
17. Kulasinghe A, Perry C, Kenny L, Warkiani ME, Nelson C, Punyadeera C. PD-L1 expressing circulating tumour cells in head and neck cancers. *BMC Cancer* 2017;**17**:4–9. http://dx.doi.org/10.1186/s12885-017-3316-3.
18. Gorges T, Pantel K. Circulating tumor cells as therapy-related biomarkers in cancer patients. *Cancer Immunol Immunother* 2013;**62**:931–9.
19. Baum B, Settleman J, Quinlan M. Transitions between epithelial and mesenchymal states in development and disease. *Semin Cell Dev Biol* 2008;**19**:292–308.
20. Borsig L, Wong R, Feramisco J, Nadeau DR, Varki NM, Varki A. Heparin and cancer revisited: mechanistic connections involving platelets, P-selectin, carcinoma mucins, and tumor metastasis. *Proc Natl Acad Sci U S A* 2001;**98**:3352–7.
21. Pantel K, Alix-Panabieres C, Riethdorf S. Cancer micrometastases. *Nat Rev Clin Oncol* 2009;**6**:339–51.
22. Meng S, Tripathy D, Frenkel EP, Shete S, Naftalis EZ, Huth JF, Beitsch PD, Leitch M, Hoover S, Euhus D, Haley B, Morrison L, Fleming TP, Herlyn D, Terstappen LW, Fehm T, Tucker TF, Lane N, Wang J, Uhr JW. Circulating tumor cells in patients with breast cancer dormancy. *Clin Cancer Res* 2004;**10**:8152–62.
23. Probert J, Thompson R, Bagshaw M. Patterns of spread of distant metastases in head and neck cancer. *Cancer* 1974;**33**:127–33.
24. Joosse S, Pantel K. Biologic challenges in the detection of circulating tumor cells. *Cancer Res* 2013;**73**:8–11.
25. Hayes DF, Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Miller MC, Matera. Allard WJ, Doyle GV, Terstappen LWWM. Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. *Clin Cancer Res* 2006;**12**:4218–25. http://dx.doi.org/10.1158/1078-0432.CCR-05-2821.
26. Amin MB, Edge S, Greene F, Byrd DR, Brookland RK, Washington MK, Gershenwald JE, Compton CC, Hess KR, Sullivan DC, Jessup JM, Brierley JD, Gaspar LE, Schilsky RL, Balch CM, Winchester DP, Asare EA, Madera M, Gress DM, Meyer LR. *AJCC cancer staging manual*. Eighth edition. Springer; 2017.
27. Kulasinghe A, Tran TH, Blick T, O'Byrne K, Thompson EW, Warkiani ME, Nelson C, Kenny L, Punyadeera C. Enrichment of circulating head and neck tumour cells using spiral microfluidic technology. *Sci Rep* 2017;**7**:42517.
28. Wang HM, Wu MH, Chang PH, Lin HC, Liao CD, Wu SM, Hung TM, Lin HY, Chang TC, Tzu-Tsen Y, Hsieh JC. The change in circulating tumor cells before and during concurrent chemoradiotherapy is associated with survival in patients with locally advanced head and neck cancer. *Head Neck* 2019;**41**:2676–87. http://dx.doi.org/10.1002/hed.25744.
29. Umeda M, Nishimatsu N, Masago H, Ishida Y, Yokoo S, Fujioka M, Shibuya Y, Komori T. Tumor-doubling time and onset of pulmonary metastasis from adenoid cystic carcinoma of the salivary gland. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1999;**88**:473–8.
30. Warkiani ME, Khoo BL, Wu L, Tay AK, Bhagat AA, Han J, Lim CT. Ultra-fast, label-free isolation of circulating tumor cells from blood using spiral microfluidics. *Nat Protoc* 2016;**11**:134–48.
31. Girelli L, Locati L, Galeone C, Scanagatta P, Duranti L, Licitra L, Pastorino U. Lung metastasectomy in adenoid cystic cancer: is it worth it? *Oral Oncol* 2017;**65**:114–8. http://dx.doi.org/10.1016/j.oraloncology.2016.10.018.
32. Hashimoto M, Tanaka F, Yoneda K, Takuwa T, Kuroda A, Okumura Y, Kondo N, Tsujimura T, Nakano T, Hasegawa S. The clinical value of circulating tumour cells (CTCs) in patients undergoing pulmonary metastasectomy for metastatic colorectal cancer. *J Thorac Dis* 2018;**10**:1569–77. http://dx.doi.org/10.21037/jtd.2018.03.05.

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