INTRODUCTION

Squamous cell carcinoma arising in Zenker's diverticulum is a rare malignancy, mostly present in the elderly. Knowledge of this condition is limited, and no therapy guidelines are available. For the first time, we describe a case of Zenker's carcinoma with molecular characterization. We performed a literature research. For the mutational analysis, we used Oncomine™ solid tumor panel and tumor mutational burden panel from Ion Torrent, Thermo Fisher Scientific. We found 66 case reports on pharyngeal pouch carcinoma in the English literature, without any evidence-based therapy guidelines or reporting on a genetic examination. As one of the main revelations of the molecular examination, the tumor exhibits a pathogenic TP53 and PTEN mutation and an intermediate overall tumor mutational burden (TMB) of 7.9 mutations per megabase. Our results show some genetic similarities with human papillomavirus negative (HPV(-)) head and neck squamous cell carcinoma (HNSCC) which suggests that well-known therapeutic strategies may be applicable for this disease.

Zenker's diverticulum is a triangle of weakness formed between the oblique fibers of the lower inferior constrictor muscle and the transverse fibers of the cricopharyngeus muscles. The etiology of the pouch in the Killian triangle (Zenker's diverticulum) includes weakness and atrophy of muscles (mainly in elderly), combined with chronic pressure due to the increased tonus of the cricopharyngeus muscle (upper esophageal sphincter), resulting in dysphagia.

The two main treatment options for Zenker's diverticulum include transcervical excision and an endoscopic approach (rigid or flexible), which may be a good alternative in select patient groups. The annual incidence of pharyngeal diverticulum worldwide is approximately 2 per 100 000, occurring after the fifth decade of life, displaying typical symptoms of dysphagia, regurgitation of undigested food, aspiration, foetor ex ore, and weight loss. Lump in the neck, bloody sputum, and a sudden worsening of dysphagia seem to occur more frequently in Zenker's carcinoma. As symptoms are not specific, malignancy in patients presenting these conditions should always be ruled out. Differential diagnostics include pharyngeal pouch and all malignant or benign tumors (eg, hypopharynx carcinoma, proximal esophagus carcinoma,
sarcomas, inflammatory pseudotumor, and lipoma) of the hypopharynx and proximal esophagus.\(^8,9\)

Squamous cell carcinoma arising in Zenker’s diverticulum is a rarity with an incidence of 0.3%-1.5% of benign diverticula\(^5,6\) with a potentially poor prognosis of 3-year survival of 39% and 5-year survival of 14%.\(^5\) The most comprehensive systematic review published by Khan et al. in 2014 highlights many specific clinical features of Zenker’s carcinoma compared to benign pharyngeal diverticula.\(^5\) Since the number of articles published on pharyngeal pouch carcinoma is limited, there are no evidence-based diagnostic and therapy recommendations. Although genome-wide profiling studies\(^10-12\) and oncological guidelines\(^13,14\) are available on HNSCC disease, a genetic comparison between Zenker’s carcinoma and HNSCC to our knowledge has never been reported. However, still today, the specific biological pathways involved in HNSCC are indeed poorly understood. Even less information is available on hypopharynx carcinoma and on rare diseases within the head and neck areas. Therefore, taking a closer look at the molecular characterization of the pharyngeal pouch carcinoma is of high importance.

## 2 | CASE REPORT

A 74-year-old patient consulted his general practitioner in September 2018 due to rapidly progressing swallowing difficulties over several weeks. He complained additionally about bad breath and bloody sputum. He had lost 5 kg over the previous few months. The patient never smoked but drank 1-2 glasses of wine twice a week for more than ten years and was otherwise healthy.

In the clinical work-up, the barium swallow showed Zenker’s diverticulum with a diameter of 7.1 cm with irregularities of the mucosa. PET scan (Figure 1A, B), MRI (Figure 1C), and endoscopy supported suspicions of malignancy. A nasogastric tube was placed to ensure adequate food intake. The biopsy confirmed the presence of squamous cell carcinoma. The clinical situation was discussed at the tumor board, and a decision for a wide excision was made. After this, a total laryngopharyngectomy with the partial resection of the proximal trachea and esophagus, and subtotal thyroidectomy with bilateral neck dissection (levels I-VII) as well as a circular reconstruction by a free anterolateral thigh flap was performed (Figure 2). Adequate margins were controlled by frozen sections.

The postoperative follow-up was uneventful. The transplant showed good viability. The barium swallow after two weeks showed no leakage. The patient tolerated peroral feeding well, and we could remove the nasogastric tube rapidly. After three weeks, we discharged the patient in good condition with safe swallowing function. According to the National Comprehensive Cancer Network (NCCN) therapy guidelines for hypopharynx carcinoma,\(^13\) a delayed postoperative percutaneous radiotherapy is recommended.

![Figure 1](https://example.com/figure1.png)

**Figure 1** A, axial and B, coronals sections of PET scan images that show an avid FDG uptake in the hypopharyngeal/esophageal region. C, Sagittal MRI illustrating the extent of the tumor in Zenker’s diverticulum.
(intensity-modulated, up to 62.5 Grays) was performed, after initial refusal by the patient. He has a good quality of life and no evidence of recurrence 15 months after the initial surgery.

3 | MATERIALS AND METHODS

Aiming to provide the best possible therapy, we searched for guidelines and reports on the mutational analysis of pharyngeal pouch carcinoma including the available online articles published in the English language until December 2019. We used the search engines Google Scholar, Embase, and PubMed and various combinations of the following keywords "Zenker carcinoma," "pharyngeal pouch carcinoma," "pharyngeal diverticulum carcinoma," "mutational analysis," "molecular analysis," "TP53 + PTEN," and "hypopharynx carcinoma." As we did not find any evidence-based support for our therapy plan, and we were interested if Zenker’s carcinoma, which is localized in the hypopharynx, could show genetic similarities with hypopharynx carcinomas or with HNSCC, we decided to perform a molecular characterization of the tumor.

After diagnostics, the written and informed consent of the patient was obtained. Our tumor board recommended a radical tumor excision which was then performed in November 2018.

The surgical resection specimen was routinely processed for diagnostics. The carcinoma was grossly examined and cut for the preparation of paraffin sections. For the mutational analysis, we used Oncomine™ solid tumor panel and tumor mutational burden panel from Ion Torrent, Thermo Fisher Scientific. As a reference to the mutational landscape in HNSCC, we worked with the GDC Data Portal (https://portal.gdc.cancer.gov),15 as well as data from The Cancer Genome Atlas (TCGA).11

Because of a lack of specific diagnostic and therapy guidelines for Zenker’s carcinoma, we used the standard NCCN guidelines for hypopharynx carcinomas.13

4 | RESULTS

The histopathological examination showed an advanced, 9 cm-sized, moderately differentiated squamous cell carcinoma of and signs of keratinization arising in Zenker’s diverticulum. The laryngeal cartilage and focally one parathyroid gland were infiltrated, but neither lymph node metastasis, lymphatic, or blood vessel infiltration nor the infiltration of nerve sheets was detected (pT4a, pN0 (0/42), cM0, L0, V0, Pn0, R0). Resection was found to be complete with adequate margins.

In the literature research, in addition to the 43 articles with 60 case reports between 1897 and December 2008 already cited in the systematic review of Khan et al.,6 we found 6 reports published in 5 articles between December 2008 and December 2019.16-20 However, neither evidence-based recommendations nor information on molecular examination were available.

The tumor mutational burden analyzed by Ion Torrent showed a mutational load of 7.9 mutations per megabase, which was classified according to an internal validation cohort for non-small-cell lung cancers with clinical response data as intermediate.21

With the next-generation sequencing analysis (NGS) Oncomine™ Solid Tumor Panel (tumor cell content: 70%), we detected two main driver mutations: the TP53 p.R213L (allelic frequency 55%) and the PTEN p.T176* (allelic frequency 39%).

Figure 3 shows our results within the context of the mutational landscape in the head and neck squamous cell carcinomas in this anatomic region (hypopharynx). A genetic predisposition syndrome was further investigated by the evaluation of the family history, which revealed unremarkably.

Consultation of the GDC Data Portal on 8 March 2020 yielded TP53 mutations in 34.5% (n = 374/1083) of all reported HNSCC cases with 292 different mutation types. This was true in 20% (n = 5/25) for the carcinomas of the hypopharynx with five mutations. PTEN mutations occurred in
The reported occurrence of TP53 mutations was interestingly higher (57%-86%10,11) in HPV (-) HNSCC.

5 | DISCUSSION

Carcinoma in Zenker's diverticulum is an exceedingly rare malignancy. Due to the rarity of reported cases, it is challenging to establish any evidence-based therapeutic guidelines. The systematic review of Khan et al. reports that more than half of 60 patients with Zenker's carcinoma whose case was described between 1896 and 2008 underwent surgical excision alone, 20% were treated by both excision and radiotherapy, 10% underwent unimodal radiotherapy, and 17% had either no definitive treatment or did not have any treatment recorded in their case. Patients with excision alone had a prolonged survival rate compared to those who received other treatment modalities. Albeit, this difference was not statistically significant (p=0.39)6. This pattern indicates that due to a lack of evidence, Zenker's carcinoma is still being treated empirically.

As hypopharynx carcinoma and Zenker's carcinoma arise of the hypopharynx, genetic similarities and the same treatment would be foreseeable. However, a confirmation of this hypothesis is needed.

To the best of our knowledge, this is the first time a genetic examination of this rare entity has been performed. Our results indicate genetic similarities to HPV (-) HNSCC. HPV (+) and HPV (-) HNSCC seem to have different processes of carcinogenesis22 which factor may explain why Feldman et al. found TP53 as the most frequently mutated gene in HNSCC with 41%, but without any occurrence of the mutation in the HPV (+) carcinomas, and with an occurrence of 57% in HPV (-) tumors.10 Similarly, according to the Cancer Genome Atlas Network TP53 mutation occurred in 86% among the HPV (-) HNSCC samples and only in one case of 36 among HPV (+) samples.11 Stransky et al. also observed an inverse correlation between HPV status and TP53 mutation.12

The literature suggests also that hypopharynx carcinomas are more frequently HPV (-) than HPV (+).23-25 These findings may speak in favor of a similar molecular origin of Zenker's carcinoma, HPV (-) HNSCC, and hypopharynx carcinoma. However, a reliable comparison between Zenker's carcinoma and hypopharynx carcinoma is challenging because the available data about the molecular origin of hypopharynx carcinomas are also limited.

Alcohol is an established risk factor in HPV (-) HNSCC22, and the association seems to be even stronger among cancers of the oropharynx and hypopharynx.26 A prolonged exposure of the mucosa due to prolonged washout in Zenker's diverticulum suggests that alcohol may also be a potential risk factor in this condition.

Tumor mutational burden (hereinafter referred to as the “TMB”) is defined as the total number of nonsynonymous mutations per coding area of a tumor genome. It was recently shown that a high tumor mutational burden is linked to a favorable outcome for immune checkpoint inhibitor therapies. Hence, these tumors often have more neoantigens that could be recognized by the antitumor immunity. However, the cutoffs for the categories "high," "intermediate," and "low" TMB are being currently defined. Definitions for clearly high
HNSCC is known to exhibit an intermediate TMB, similarly with our case, which shows a mutational load of 7.9 mutations per megabase.

TP53 and PTEN are included in the top 11 of the most mutated genes in HPV(-) HNSCC. Somatic changes involving the gene encoding for p53 have been discovered in more than 50% of human malignancies, and both genes are considered as driving mutations.29,30

PTEN is a phosphatase involved in the negative regulation of the mitogenic RTK/PI3K/Akt pathway, thus controlling cell survival, proliferation, and growth. PTEN inhibits PI3K, whereas a mutated PTEN gene, the presence of a pseudogene or other complex downregulation mechanisms, leads to decreased or inactive PTEN protein expression, thus PI3K overexpression and as such favors the proto-oncogene MYC’s action on cell cycle and survival.30-32 This mechanism is responsible for the development of various tumors; the mutation has been described in 35% of uterine, 17% of the brain, 7.5% of bronchus and lung, and in 6.7% of breast cancers.15

PTEN mutations occurred in 2%-33% of HNSCC, and the lack of PTEN expression seemed to be linked to a poor disease-free and overall survival rates.30,33

The occurrence of PTEN mutations in hypopharynx carcinomas was not reported, and the consultation of the GDC portal did not yield any PTEN mutations in this condition.15

However, there are pieces of evidence on the crucial tumor suppressor role of PTEN in hypopharynx carcinomas. Wang et al., for example, were able to inhibit cell proliferation and malignant progression of hypopharyngeal carcinoma by overexpressing MicroRNA-98 and so activating the PTEN/AKT pathway.34

Synergies between both tumor suppressor pathways are described since both genes are involved in the regulation of cell cycle and survival. P53 and PTEN work together in feedback loops, regulating each other by shared interactor proteins. P53 seems to upregulate PTEN transcription by binding an upstream element on the PTEN promoter.32 It was also shown that the PI3K/Akt pathway promotes the movement of the Mdm2 oncoprotein into the nucleus, where it downregulates p53. PTEN inhibits PI3K/Akt, promoting p53 function.35 However, wild PTEN can enhance the gain-of-function of mutant p53 protein levels.36

Snietura et al. found PTEN to be of prognostic utility in postoperative radiotherapy in HNSCC. Since mutations in PTEN mostly lead to decreased protein expressions, it may indicate a worse prognosis in HNSCC patients. TP53 mutations seen predominantly in HPV (-) HNSCC are also associated with worse overall survival.37

This indicates similarities with the poor prognosis of Zenker’s carcinoma published by Bowdler et al.5 which may be explained by the fact that the diagnosis is generally made at advanced stages, but probably also by the disadvantageous molecular pattern revealed in our case. The molecular examination of Zenker’s carcinoma is crucial because of its bad prognosis and because the disease is frequently advanced by the time of the diagnostics. In these situations, chemotherapy and emerging biological therapies play an important role. In case of metastasis and/or lymph node affection, a systematic therapy (depending on the disease combined with radiotherapy) is administered.33 Actual standards for the systemic therapy of very advanced HNSCC (including hypopharynx carcinoma) include chemotherapy with cisplatin or carboplatin, cetuximab, paclitaxel, hydroxyurea, 5-fluorouracil, depending on the regimen. For recurrent/metastatic disease, biological therapies with anti-programmed death 1 (PD1) antibody nivolumab or pembrolizumab may be used.14 It was shown that TP53-mutated carcinomas frequently develop chemotherapy resistance.38,39 This mechanism can be inhibited by several molecules (eg, MIRA1 and PRIMA1 reactivate mutant p53 and nutlins inhibit Mdm2)39, but the topic is under continuous investigation and these agents are to the best of our knowledge not yet approved.

The information about PTEN mutations seems to also have some benefits; if we think about the study of Wang et al.34, Zenker’s carcinoma may be a future target of one of these promising biological therapies.

Taken together, the revealed molecular similarities between Zenker’s carcinoma and HPV(-) HNSCC suggest that therapeutic strategies may be applicable to this rare entity. As both hypopharynx and Zenker’s carcinoma arise of the hypopharynx, and most hypopharynx carcinomas are HPV (-), similar pathogenesis and etiology are even more suggestive. However, for a conclusive genetic comparison, more data would be necessary, and this could prove difficult due to the rarity of the disease. On the other hand, revealing the mutations may open some new possibilities for future targeted therapies in this condition.
CONSENT STATEMENT

Written and informed consent of the patient was obtained.

ACKNOWLEDGMENTS

Published with written consent of the patient.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Z.N. and L.M.: involved in literature research and manuscript writing; M.B., S.H.: involved in histology, molecular analyses, manuscript writing; in “results”—histopathology and molecular examination. All authors contributed to the critical review of the manuscript.

ORCID

Zsanett Noel https://orcid.org/0000-0002-2962-367X

REFERENCES

1. Killian G. Über den Mund der Speiseröhre. Zeitschrift für Ohrenheilkunde. 1908;55:51–41.
2. Zenker FAVZH. Krankheiten des Oesophagus. In: Handbuch der Speziellen Pathologie und Therapie. Leipzig: FC Vogel; 1877:1–87.
3. Peters JH, Mason R. Die pathophysiologische Basis des Zenker-Divertikels. Der Chirurg. 1999;70(7):741–746.
4. Shahawy S, Janisiewicz AM, Annino D, Shapiro J. A comparative study of outcomes for endoscopic diverticulotomy versus external diverticulectomy. Otolaryngol Head Neck Surg. 2014;151(4):646–651.
5. Bowdler DA, Stell PM. Carcinoma arising in posterior pharyngeal pulsion diverticulum (Zenker’s diverticulum). Br J Surg. 1987;74(7):561–563.
6. Khan AS, Dwivedi RC, Sheikh Z, et al Systematic review of carcinoma arising in pharyngeal diverticula: A 112-year analysis. Head Neck. 2014;36(9):1368–1375.
7. Mirza S, Dutt SN, Minhas SS, Irving RM. A retrospective review of pharyngeal pouch surgery in 56 patients. Ann R Coll Surg Engl. 2002;84(4):247–251.
8. Graefe H, Stellmacher F, Sotlar K, Wollenberg B, Gehring E. Inflammatory pseudotumor of the hypopharynx: clinical diagnosis, immunohistochemical findings and treatment of this rare disease. Vivo. 2008;22(6):817–820.
9. Oliver Kaschke HB. In: Ear, Nose, and Throat Diseases: With Head and Neck Surgery. Stuttgart, New York Thieme; 2009:280.
10. Feldman R, Gatalica Z, Knezetic J, Reddy S, Nathan CA, Javadi N, Teknos T. Molecular profiling of head and neck squamous cell carcinoma. Head Neck. 2016;38(S1):E1625–E1638.
11. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature. 2015;517(7536):576–582.
12. Stransky N, Egloff AM, Tward AD, et al The mutational landscape of head and neck squamous cell carcinoma. Science. 2011;333(6046):1157–1160.
13. https://nccn.org/NCCNguidelinesfortreatmentofcancerbysite/HeadandNeckCancers
14. Oosting SF, Haddad RI. Best practice in systemic therapy for head and neck squamous cell carcinoma. Front Oncol. 2019;9:815.
15. Head and Neck/ squamous cell carcinoma/ Gene: TP53. https://portal.gdc.cancer.gov.
16. Acaț T, Savas R, Kocacelebi K, Ersoz G. Squamous cell carcinoma arising from a Zenker’s diverticulum: contribution of FDG-PET/CT to the diagnosis. Dis Esophagus. 2016;29(8):1170–1171.
17. Khan AS, Dwivedi RC, Khan AS, Thway K, Fisher C, Rhys-Evans PH. Carcinoma arising from pharyngeal diverticula. Head Neck. 2011;33(1):135–137.
18. Ohara T, Shirakawa Y, Noma K, et al Direct observation diverticulectomy for Zenker’s diverticulum carcinoma. Esophagus. 2013;10(4):235–238.
19. Hajjar WM, Almutairi OT, Jameel MA, Al-Nassar SA, Raddaoui E, Hajjar AW. Squamous Cell Carcinoma Arising in Zenker’s Diverticulum: A Case Report and Review of the Literature. Turk Thorac J. 2018;1–3.
20. Fox SS, Nagasawa KK, Williams ZF. Incidental Squamous Cell Carcinoma In Situ in a Large Pharyngoesophageal (Zenker’s) Diverticulum. Am Surg. 2017;83(8):e288–e290.
21. Melendez B, Van Campenhout C, Rorive S, Remmelink M, Salmon I, D’Haene N. Methods of measurement for tumor mutational burden in tumor tissue. Transl Lung Cancer Res. 2018;7(6):661–667.
22. Arenz A, Patze J, Kormann E, et al HPV-negative and HPV-positive HNSCC cell lines show similar numerical but different structural chromosomal aberrations. Head Neck. 2019;41(11):3869–3879.
23. Dahm V, Haitel A, Kaidar A, Stanisz I, Beer A, Lill C. Cancer stage and pack-years, but not p16 or HPV, are relevant for survival in hypopharyngeal and laryngeal squamous cell carcinomas. Eur Arch Otorhinolaryngol. 2018;275(7):1837–1843.
24. Wendl M, Romanian M, Näsman A, et al Presence of human papillomaviruses and p16 expression in hypopharyngeal cancer. Head Neck. 2014;36(1):107–112.
25. Sivars L, Bersani C, Grün N, et al The Human papillomavirus is a favourable prognostic factor in cancer of unknown primary in the head and neck region and in hypopharyngeal cancer. Mol Clin Oncol. 2016;5(6):671–674.
26. Kawakita D, Matsu K. Alcohol and head and neck cancer. Cancer Metastasis Rev. 2017;36(3):425–434.
27. Maleki VS. High and low mutational burden tumors versus immunologically hot and cold tumors and response to immune checkpoint inhibitors. J Immunother Cancer. 2018;6(1):157.
28. Chalmers ZR, Connelly CF, Fabrizio D, Gay L, et al Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med. 2017;9(1):34.
29. Perri F, Piscinto S, Della Vittoria Scarpati G. PS5 mutations and cancer: a tight linkage. Ann Transl Med. 2016;4(24):522.
30. Squarize CH, Castilho RM, Abrahao AC, Molinolo A, Lingen EM. PTEN/PTENP1: ‘Regulating the regulator of RTK-dependent PI3K/Akt signalling’, new targets for cancer therapy. Mol Cancer. 2015;14(7):189.
31. Snietura M, Jaworska M, Mlynarczyk-Liszka J, et al PTEN as a prognostic and predictive marker in postoperative radiotherapy for squamous cell carcinomas of the head and neck region and in hypopharyngeal cancer. Head Neck. 2016;38(1):107–112.
32. Haddadi N, Lin Y, Travis G, Simpson AM, Nassif NT, McGowan EM. PTEN/PTENP1: ‘Regulating the regulator of RTK-dependent PI3K/Akt signalling’, new targets for cancer therapy. Mol Cancer. 2015;14(7):37.
33. Di Cristofano A, Pandolfi PP. The Multiple Roles of PTEN in Tumor Suppression. Cell. 2000;100(4):387–390.
34. Wang Q, Tan L, Liu J, Zhao J, Zhou X, Yu T. MicroRNA98/PTEN/AKT pathway inhibits cell proliferation and malignant progression of hypopharyngeal carcinoma by MTDH. Oncol Rep. 2019;41(2):863–874.

35. Mayo LD, Donner DB. The PTEN, Mdm2, p53 tumor suppressor-oncoprotein network. Trends Biochem Sci. 2002;27(9):462–467.

36. Li Y, Guessous F, Kwon S, et al PTEN has tumor-promoting properties in the setting of gain-of-function p53 mutations. Cancer Res. 2008;68(6):1723–1731.

37. Zhou G, Liu Z, Myers JN. TP53 mutations in head and neck squamous cell carcinoma and their impact on disease progression and treatment response. J Cell Biochem. 2016;117(12):2682–2692.

38. Zhou X, Hao Q, Lu H. Mutant p53 in cancer therapy—the barrier or the path. J Mol Cell Biol. 2019;11(4):293–305.

39. Hientz K, Mohr A, Bhakta-Guha D, Efferth T. The role of p53 in cancer drug resistance and targeted chemotherapy. Oncotarget. 2017;8(5):8921–8946.

40. p53.iarc.fr: Home / Resources and links / TP53 sequences/ Graphical view of the Genomic sequence (hg38) with exon-intron boundaries for the main p53 isoform.

41. Campo E, Cymbalista F, Ghia P, et al TP53 aberrations in chronic lymphocytic leukemia: an overview of the clinical implications of improved diagnostics. Haematologica. 2018;103(12):1956–1968.

How to cite this article: Noel Z, Hoeller S, Bihl M, Muller L. TP53 and PTEN as driver mutations in Zenker’s carcinoma—a clinical presentation. Clin Case Rep. 2020;8:2790–2796. https://doi.org/10.1002/ccr3.3169