Dear Editor,

We thank the authors of the letters for their constructive comments and thereby their contribution to the interpretation of the glandular structure as described in the tubarial gland paper and the clinical consequences of its presence [1]. Their comments will be discussed by topic in this summarized reply. Our reply to the comments in a point-by-point response to each letter is provided in the Supplemental material.

The glands clustered in the mucosa of the auditory tube and regions adjacent to the tubarius torus have been earlier described by anatomists

The words “new” and “organ” in the title seem to have been a trigger for massive media attention [4]. This might have caused some distraction from the core message of the manuscript. We therefore thank Mudry and Jackler for their view on the focus of our paper: “reclassification of a well-known anatomical feature rather than a striking discovery of an unrecognized structure somehow overlooked by generations of anatomists”, and its possibly important role in preserving salivary gland function in radiotherapy for head and neck cancer [4]. It is indeed important to emphasize that clusters or a high density of seromucous glands in this area have been described before. The authors of the original paper realized that they were standing on the shoulders of giants in this area. For that reason, we examined commonly used anatomical atlases and reviewed available papers in English and non-English from the past two centuries, of which the oldest was a German paper by Moos, dating back to 1874 [15]. Some (but not all) of these giants in anatomy were mentioned in the reference list of our paper and they referred to the even older papers of e.g. Moos [2,3].

The core of our message is the fact that the gland tissue in this area has not been considered as an organ at risk (OAR) in radiotherapy. One should realize that an OAR does not only concern organs, but it comprises all tissues near the clinical target volume that can get damaged by radiation. The new techniques of PSMA PET/CT and 3D reconstruction of histological slides that were presented in the paper, opened a new perspective that did not fit previous anatomical descriptions. To our knowledge, there has not been a prior interpretation of the gland tissue in this area as a localized and organized macroscopic glandular structure, similar to one of the major salivary glands (the sublingual gland). Earlier contributions by colleagues to the knowledge of the embryology and anatomy of the nasopharynx fit into this new perspective, but in our opinion, it is not the other way around.

Therefore this bilateral gland structure that we named the “tubarial glands” can be regarded as an OAR. Earlier, this realization led to our evaluation of toxicity in a cohort of 723 head and neck cancer patients, in which that theory was confirmed.

As far as the anatomical classification is concerned, we took a rather neutral position on the interpretation of findings and wrote in the discussion section: “Regardless of the classification of the tubarial glands as either a conglomerate of minor glands, a major gland, a separate organ, or as a new part of an organ system, the tubarial glands are macroscopic gland tissue locations with clinical relevance”. We thank Nascimento et al., Iwanaga et al., Mudry et al., Goldemberg et al. and Narayan et al. for their comments regarding the aspects mentioned above [4–7][14].

It is too early to call this gland a salivary gland

We thank Bikker and Vissink for their constructive input concerning the interpretation of the glandular structure that was described in the tubarial gland paper [1,8]. They argue that it is too early to call this gland a salivary gland for several reasons.

First, the question is raised whether the acinar cells actually produce saliva, as the authors of the letter seem to have set this as a criterium for the glands to be called salivary glands. The problem in answering that question, is that saliva is not strictly defined, as all fluids that contribute to the fluid in the oral cavity together are called saliva. These fluids are produced in the major (parotid, submandibular and sublingual) and minor (spread out microscopic) salivary glands and gingival crevice and have widely ranging compositions [9]. As an example, the amylase concentration varies by tenfold between the parotid and sublingual gland and the uncontaminated crevicular fluid (formed by serum and locally generated materials) contains no amylase [9–11]. Therefore, in our opinion, the uninvestigated fluid composition argument to call the tubarial glands salivary glands does not seem to hold.

Second, Bikker and Vissink argue that the tubarial glands do not contribute to the oral fluid, but they do not provide evidence for this statement. In addition, one could question this criterium for calling the tubarial glands actual salivary glands. Functional and histological characteristics rather than location of the gland or its fluid, should determine whether it can be considered a salivary gland. However, we have been able to perform a simple in vivo pilot study (n = 1) in a healthy volunteer, in which 1 ml of blue

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dye in thickened slimy saline was put directly next to the tubarial gland on the cranial side of the soft palate. This subsequently led to staining of the pharyngeal wall, but also of the palatopharyngeal arch, which is the dorsal border of the oral cavity (Fig. 1 in the Supplementary material). Based on this finding, one could say it is not unthinkable that the excreted fluid from the tubarial glands reaches the oral cavity. If this contribution to the oral cavity fluid is used as a criterium for saliva, the fluid from the tubarial glands might thus be considered as saliva.

Third, the authors of the letter mention the absence of a main excretory draining duct as an argument against the categorization as a major gland, but they at the same time admit that this is not really an argument, because part of the sublingual gland has similar ducts as the tubarial glands. The relativity of this argument was already discussed in the original paper. Additionally, the excretory duct openings of the tubarial glands are macroscopically visible as small holes filled with a secrete (see Fig. 2B and 2C in the original paper), in contrast to and larger than labial duct openings.

Finally, Bikker and Vissink mention that salivary glands are known to secrete amylase. This was shown by Veerman et al. who investigated secreted saliva that was collected from the parotid, submandibular and sublingual glands [9]. We investigated tubarial gland cells by immunohistochemistry (IHC), instead of collecting excreted fluid because of the risk of contamination by saliva of other glands. Our findings by IHC showed that amylase was

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**Fig. 1.** Torus tubarius (A). Thickened saline with blue dye applied right next to the tubarial gland area (B). Blue dye on the palatopharyngeal arch, which is part of the oral cavity (C).

**Fig. 2.** Alcian blue staining of the auditory tube deep to the tubarial gland, showing mucin in the glands of the pharyngeal wall, near and possibly as part of the tubarial gland.

**Fig. 3.** Amylase immunohistochemistry: tubarial gland (A), palatal glands (B), parotid gland (C), sublingual gland (D).
expressed in neither the tubarial, nor the palatal and sublingual glands, in contrast to expression in the parotid gland (Fig. 3 in the Supplementary material). Since these three gland locations thus did not differ using this sensitive method, there was no urgent reason to measure other proteins or enzymes as is suggested by the authors of the letter. However, an Alcian blue staining was performed for the 3D reconstruction slides, showing presence of mucin in the gland cells of the pharyngeal wall next to the auditory tube (Fig. 2 in the Supplementary material). Further characterization of the tubarial glands by its protein excretion profile and stimuli for excretion as suggested, is however thankfully welcomed to provide more insight. The original authors invite the authors of the letter to do so in a constructive cooperation.

If the newly described gland structures have a similar PSMA-expression profile, similar histology as the sublingual glands, with a similar amylase absence on IHC, similar multiple draining ducts, and a similar profile of benign and malignant tumors, according to the WHO International Classification of Diseases for Oncology (ICD-O), one could say there are sufficient arguments to call these glands salivary glands. But we do agree that further characterization of the tubarial gland fluid and its course downwards through the pharynx and over the dorsal tongue would provide more insight in its function and contribution to the saliva in the oral cavity.

In conclusion, we think that the arguments in favor of a classification of the tubarial glands as salivary glands outperform the arguments against it. We again emphasize the fact that the major/minor gland classification system has its limitations. However, we thankfully endorse the suggestions that were made for further characterization of this newly interpreted part of the salivary glands system, not only for academical reasons, but also to better understand the potential benefit for head and neck cancer patients.

No convincing evidence for the presence of tubarial salivary glands

We thank Iwanaga et al. for their interpretation and comments regarding the presented evidence [6]. They state that the existence of the tubarial salivary glands is not proven for three reasons. First, PSMA PET/CT does not prove existence of a salivary gland. Second, histology does not prove existence of a salivary gland. Finally, it is anatomically incorrect to say this represents a newly discovered salivary gland.

This interpretation is not in agreement with statements in the original paper, as we show in the point-by-point response in the Supplementary material.

We thank Narayan et al. for forth bringing their interpretation of our paper [14]. Similar to the points mentioned earlier in this response to the letters, it interestingly shows that important nuances in the discussion section regarding interpretation of the findings by the original authors, seem not to have been noted by Narayan et al. Also, the radiotherapy term organ at risk (OAR) seems to have been misinterpreted as the anatomical term “organ”. These misunderstandings might have resulted from the monodisciplinary anatomical background of the authors of the letter as in contrast to the multidisciplinary (7 specialty) team of authors of the original paper. The suggestions for further characterization are, however, thankfully taken. As is said in the title of our response, the original paper is a starting point.

The study design created considerable gender imbalance, with possible clinical consequences

We thank the authors Ellsworth et al. for their remarks on gender imbalance. We should have realized and mentioned these concerns in the limitations section [12]. To provide more insight in the female tubarial glands, we have now been able to examine six additional female patients that were scanned with PSMA PET/CT for another study. We can report that all scanned women had the expected visual indications of tubarial glands, with a median cranio-caudal length of 3.3 cm (range 2.2–4.6 cm). This number is too low for an actual statistical comparison between the sexes, but it is comparable to the length of the gland in the 99 men in the paper: median 3.9 cm (range 1.0–5.7 cm).

There are more clinical concerns than only in radiotherapy

We thank Thakar et al. for their contribution regarding the clinical implications of the findings reported in our paper [13]. The realization of the presence of these relatively large glandular structures might indeed have clinical consequences in more (aspects of) diseases than we described in our paper. They report diagnostic concerns in juvenile nasal angiofibroma (JNA) evaluated by PSMA PET/CT. Also, pathological concerns are mentioned in diagnosing tumors in the nasopharynx that should take the tubarial glands into account. But perhaps most importantly regarding our paper, they emphasize the relevance for head and neck radiotherapy and suggest routinely implementation as an organ at risk. The authors of the original paper made recommendations regarding implementation: “We prefer to acquire external validation in an independent dataset and advise to change clinical protocols only in the setting of continued monitoring of anticipated clinical benefits”. For this reason, we referred to our original paper as “a starting point” in the title of this reply to the comments in 8 letters-to-the-editor.

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Appendix A. Supplementary data

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References


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