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## Clinical management of salivary gland hypofunction and xerostomia in head and neck cancer patients: successes and barriers\*

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### Abstract

The most significant long-term complication of radiotherapy in the head and neck region is hyposalivation and its related complaints, particularly xerostomia. This paper addresses the pathophysiology underlying irradiation damage to salivary gland tissue, the consequences of radiation injury, and issues contributing to the clinical management of salivary gland hypofunction and xerostomia. These include ways to: (1) prevent or minimize radiation injury of salivary gland tissue, (2) manage radiation-induced hyposalivation and xerostomia, and (3) restore the function of salivary gland tissue damaged by radiotherapy.

### Keywords

radiotherapy; hyposalivation; xerostomia; prevention; palliative care; gene transfer; stem cell therapy

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## Introduction

Radiotherapy plays a pivotal role in the curative treatment of the majority of patients with head and neck cancer, either as single modality or in combination with surgery and/or chemotherapy. Despite the beneficial effects of radiotherapy in loco-regional tumor control, the damage inflicted to normal tissues surrounding the tumor may cause severe complications. In particular, co-irradiation of the salivary glands during the treatment of head and neck cancer results in a progressive loss of gland function (hyposalivation) beginning early in the course of radiotherapy.<sup>1</sup>

### Oral sequelae resulting from radiation injury of salivary gland tissue

Quantitative and qualitative salivary changes predispose the irradiated patient to a variety of problems that develop either directly or indirectly as a result of diminished salivary output.<sup>2-4</sup> These include oral dryness, impairment of normal oral functions (speech, chewing and swallowing) because of insufficient wetting, and decreased lubrication of the mucosal surfaces and of ingested food. Furthermore, the oral mucosa can become dry and atrophic, leading to frequent ulceration and injury. Finally, the shift in oral microflora towards cariogenic bacteria, the reduced salivary flow (oral clearance), and changes in saliva composition (decreased buffer capacity, pH, immunoprotein concentrations), may result in rapidly progressing radiation caries.<sup>2,5</sup>

Although most studies focus on salivary flow, other endpoints related to salivary function, such as patient-rated xerostomia and physician-rated RTOG xerostomia, are probably of even more clinical relevance.<sup>6,7</sup> Importantly, the subjective symptom of xerostomia may not always correlate with salivary flow rates. For understanding this phenomenon, one should be aware that saliva enters the mouth at several locations, and that the different glandular secretions are not well mixed.<sup>8</sup> For example, the contribution of parotid saliva to (un)stimulated whole saliva varies from site to site, ranging from being the major contributor to whole saliva collected buccally from the maxillary molars to being almost non-contributing to whole saliva collected in the incisor region.<sup>9</sup> The wide variation in local contribution of the various salivary glands to whole saliva is also obvious when assessing mucosal wetness as the thickness of the salivary layer on the oral mucosa is much thinner in the labial and anterior hard palatal region than on the buccal mucosa and anterior tongue.<sup>10</sup> These phenomena might explain, at least in part, the differences reported in the literature about level of hyposalivation and sensation of oral dryness.

### Pathophysiology of radiation damage to salivary gland tissue

Radiation-induced DNA damage impairs proper cell division, resulting in cell death or senescence of cells that attempt to divide. Based on the slow turnover rates of their cells (60–120 days), the salivary glands would be expected to be late responding tissue (>60 days) (Fig. 1).<sup>11</sup> However, the changes in quantity and composition of saliva that occur shortly after radiotherapy indicate that these glands respond acutely.<sup>1,12</sup> Radiation injury leads primarily to the loss of saliva-producing acinar cells, however interestingly, the ducts, although deprived of function, mostly remain intact.<sup>13</sup> A human postmortem study suggests that in the lower dose range (< 30 Gy, in 2 Gy fractions) damage is reversible to a certain extent, but with cumulative doses (> 75 Gy) extensive degeneration of acini is observed along with inflammation and fibrosis in the interstitium.<sup>14</sup> The role of apoptotic cell death in early salivary gland dysfunction after radiotherapy remains unclear. Paardenkooper *et al.*<sup>15</sup> did not observe a dose-related increase in apoptotic cells very early after radiotherapy, while Avila *et al.*<sup>16</sup> found that early radiation-induced salivary gland dysfunction resulted from p53-dependent apoptosis.

Next to the suggestion of massive apoptosis, the leakage of granules and subsequent lysis of acinar cells has been suggested as an alternative explanation for the acute radiation-induced dysfunction of the salivary glands.<sup>17,18</sup> However several studies show no cell loss during the first days after irradiation, although saliva flow is dramatically reduced, and water secretion is selectively hampered.<sup>19–22</sup> One mechanism of action to explain the early effects and the enigmatic high radiosensitivity of salivary cells is selective radiation damage to the plasma membrane of the secretory cells resulting in disruption of muscarinic receptor-stimulated water secretion. Based on their studies in the rat model, Coppes *et al.*<sup>21</sup> have proposed that radiation-induced loss of salivary gland function occurs over four phases. The first phase (0–10 days) is characterised by a rapid decline in flow rate without changes in amylase secretion or acinar cell number. The second phase (10–60 days) consists of a decrease in amylase secretion paralleled by acinar cell loss. Flow rate, amylase secretion and acinar cell numbers do not change in the third phase (60–120 days). In the fourth phase (120–240 days) further deterioration of gland function is seen, but is accompanied by an increase in acinar cell number, albeit with poor tissue morphology. Comparable changes have been observed in rat submandibular tissue; however similar studies are not available in humans.<sup>12,22</sup>

## Prevention of radiation-induced injury to the salivary gland

In humans, depending on the localization of the radiation portals, a rapid decrease of the salivary flow rate is observed during the first week of radiotherapy, after which there is a continuing gradual decrease to less than 10% of the initial flow rate (Fig. 2).<sup>1,23,24</sup> Although in the older literature the submandibular gland was thought to be less radiosensitive than the parotid gland, both glands have been shown to be as sensitive to radiotherapy, at least with respect to their function.<sup>1,23,24</sup> The range of the mean doses, which represents the threshold for significant salivary flow reduction, is 26 to 39 Gy.<sup>25–28</sup> Recently, it was shown that there is no threshold dose for reduced parotid gland function after radiotherapy and the TD50 is probably equal to 40 Gy,<sup>29</sup> comparable to the TD50 (39 Gy) for submandibular gland function.<sup>26</sup>

## Advances in radiation delivery

**Intensity modulated radiotherapy (IMRT)**—Changing a conventional schedule of fractionated radiotherapy into a schedule of continuous, hyperfractionated, accelerated radiotherapy (CHART) results in some sparing of salivary gland function; however IMRT allows a more accurate delivery of specific radiation dosage and dose distribution to the tumor and thereby brings about the possibility of better sparing of surrounding tissues, e.g. major salivary glands. IMRT is currently recommended as a standard approach in head and neck cancer to limit the cumulated radiation dose to critical normal tissues. IMRT can reduce the dose to parotid, submandibular/sublingual and minor salivary glands while helping maintain adequate whole saliva flow rates and reducing xerostomia (Fig. 2)<sup>6,25–27</sup>. Although IMRT, when compared to two-dimensional radiotherapy (2D-CRT), results in a significant reduction of patient- and observer-rated xerostomia, still about 40% of patients complain of xerostomia.<sup>30</sup>

**Proton radiotherapy**—Further improvement of salivary gland tissue sparing could be achieved by new radiation techniques using charged particles, e.g., protons instead of the currently used photons. The physical and radiobiological properties of protons allow a superior dose distribution as compared to current photon (X-ray) radiotherapy, thereby minimizing the dose to normal tissues and significantly reducing acute and late side effects. The potential benefit of protons have been investigated in a number of *planning comparative* studies that show that the dose to critical organs can be significantly reduced when protons are used, particularly for tumors originating in the pharynx,<sup>31,32</sup> the paranasal sinuses<sup>33,34</sup>

and in head and neck cancer patients treated with bilateral neck irradiation.<sup>35</sup> In some studies, a significant reduction of the risk of side effects was observed in approximately 70% of the cases based on existing and validated NTCP (normal tissue complication probability) models.<sup>35</sup>

## Radioprotectors

**Amifostine**—Direct radioprotection may be achieved by the use of amifostine, an oxygen radical scavenger, when systemically administered during radiation treatment (Table 1).<sup>36,37</sup> Although amifostine has the potential to reduce xerostomia during and post radiation treatment, a significant proportion of patients continue to experience xerostomia.<sup>40</sup> Intravenous administration of amifostine is accompanied by many side effects; recently it has been shown that subcutaneous administration of amifostine provides a well-tolerated alternative.<sup>41</sup> Unfortunately, amifostine might have the undesirable effect of tumor protection, thus the controversy continues as to whether amifostine is safe for use in cancer patients.<sup>37,42</sup> Guidelines for the use of amifostine and other agents are presented in Table 1.

**Tempol**—Tempol, is a stable nitroxide, is thought to provide radioprotection by several mechanisms including oxidizing transition metals, mimicking superoxide dismutase activity, and scavenging free radicals. In a mouse model it was shown that administration of Tempol i.p., i.v., s.c. or even in topical form (mouthwash, gel), all significantly reduced irradiation-induced salivary gland hypofunction.<sup>43,44</sup> Moreover, Tempol provided salivary gland protection but did not protect tumor tissue.<sup>45</sup> These various studies support further development and consideration of Tempol for human clinical trials as a selective protector against radiation-induced salivary gland damage.

**Other preventive agents**—Other potential preventive agents include pre- or during radiotherapy administration of insulin growth factor 1 (IGF-1) or keratinocyte growth factor (KGF), both of which have been studied in mice. The rationale for use of growth factors is to suppress apoptosis and/or enhance survival and proliferation of salivary acinar cells post-radiation.<sup>16,46</sup> Utilization of IGF-1 has been shown to reduce radiation-induced apoptosis and preserve salivary gland function in a mouse model extending the causal relationship between apoptosis and dysfunction.<sup>46</sup> Subcutaneous administration of KGF, either before or directly after irradiation also reduced radiation-induced hyposalivation.<sup>47</sup> Post-irradiation administration of KGF seemed to act through accelerated expansion of the pool of progenitor/stem cells that survived the irradiation treatment. Recently, it was shown that pre-irradiation intraglandular administration of botulinum toxin into rat submandibular glands can also significantly reduce radiation injury at a glandular level.<sup>48</sup>

## Strategies for restoring salivary gland function following irradiation injury

### Stimulation of residual function

Administration of pilocarpine or pure cholinergic sialogogues to stimulate any residual function of the salivary gland post-radiotherapy is worthwhile (Tables 1 and 2), however the functional gain ceases as soon as the administration of the sialogogue is stopped.<sup>49,50</sup> A more persistent effect can be observed when pilocarpine is administered before radiotherapy and continued during radiotherapy and then stopped.<sup>38</sup> Moreover, in a rat study it was shown that amelioration of early loss of rat salivary gland function after radiation by pilocarpine pretreatment was, at least in part, due to compensatory mechanisms through increased proliferation of undamaged cells.<sup>51,52</sup> In a large study by Scarantino *et al.*,<sup>53</sup> no significant differences in xerostomia, mucositis and quality of life were found, but unstimulated whole salivary flow significantly increased. In the study of Burlage *et al.*<sup>38</sup> no statistically significant differences between the pilocarpine and the placebo group could be

found overall. However, the results of that study also showed that the beneficial effect of pilocarpine depended on the dose distribution in the parotid glands, indicating that when the mean parotid dose exceeds 40 Gy, pilocarpine might significantly spare parotid flow and reduce patient-rated xerostomia, particularly after 12 months.

Probably, a significant part of the beneficial effect of pilocarpine on post-irradiation xerostomia can be attributed to stimulation of the minor salivary glands as the mucous minor palatal glands have been shown to have a greater resistance to irradiation than serous parotid glands.<sup>54</sup> Other drugs that have been reported to be of significance in the treatment of hyposalivation include anetholtrithione<sup>55</sup> and cevimeline.<sup>56</sup> As the stimulating properties of these sialogogues are short-lived, patients have to use them for the rest of their lives.

### Acupuncture

Stimulation of residual salivary secretory capacity by acupuncture has shown some promising results in head and neck radiotherapy patients (Table 1).<sup>57–59</sup> The effects of acupuncture treatment on secretion of whole saliva and related symptoms are sustained for at least six months<sup>57,59,60</sup> and additional acupuncture therapy can maintain this improvement up to 3 years.<sup>57</sup> Moreover, Deng *et al.*<sup>61</sup> showed that acupuncture was associated with neuronal activation that was absent during sham acupuncture stimulation.

### Oral lubricants and saliva substitutes

When stimulation of residual secretion is insufficient the clinician is left with a purely symptomatic approach. This is most commonly through the frequent use of water, although complex saliva substitutes have been developed that contain agents to impart viscosity and to keep soft tissues moist (Table 2). These substitutes are either based on carboxymethylcellulose (CMC)<sup>62</sup>, mucin<sup>63</sup> or xanthan gum<sup>64</sup>. Mucin-containing and xanthan gum-containing saliva substitutes are usually preferred over CMC-containing substitutes, both by patients with Sjögren's syndrome and radiation-induced xerostomia as they have superior rheological and wetting properties.<sup>64,65</sup> In addition 'gel-like' saliva substitutes have been developed, of which the polyglyceryl methacrylate based substitute holds promise,<sup>66</sup> particularly when employed at night and when daily activities are at a low level. It is worthwhile to try different types of saliva substitutes in a particular patient in order to select the most effective substitute for that patient.<sup>64,67</sup> Based on the literature, recommendations for the treatment of hyposalivation have been summarized in Table 3.<sup>66</sup>

## Promising new approaches for restoration of salivary gland function

### Gene therapy

In the future gene therapy might provide a therapeutic option for radiation induced-salivary hypofunction in some patients. The gene transfer strategy pioneered by Baum *et al.*<sup>68</sup> focused on developing a gene transfer event that could elicit fluid secretion from surviving (primarily duct) epithelial cells in an irradiated salivary gland.<sup>69</sup> Delporte *et al.*<sup>69</sup> reasoned that surviving duct cells could serve as water secreting cells if there was a pathway for water transport inserted in the duct cell membranes. Their approach utilized the water channel protein, human aquaporin-1 (hAQP1).<sup>70</sup> hAQP1 can facilitate the extremely rapid movement of water in response to an osmotic gradient, and expression of hAQP1 protein in cell types in which it is not normally found can lead to dramatic increases in osmotically-obliged water movement (Fig. 3a). There have been two key pre-clinical studies with hAQP1. Both utilized a recombinant serotype 5 adenoviral vector encoding hAQP1, AdhAQP1, which was delivered to salivary glands via intraductal cannulation through the orifice of the main excretory duct.<sup>71</sup> In the first *in vivo* study irradiated rats given AdhAQP1 displayed salivary flow rates approaching those of sham-irradiated animals treated with the



control virus, i.e., nearly normal.<sup>69</sup> The second key *in vivo* study was a longitudinal study and targeted parotid glands in a large animal, the miniature pig (~25–30kg).<sup>72</sup> Sixteen weeks following irradiation, salivary secretion was decreased by >80%. Administration of AdhAQP1 resulted in a dose-dependent increase in parotid salivary flow to ~80% pre-irradiation levels on day 3 (Fig. 3b). Since the hAQP1 transgene was expressed only in parotid duct cells, the implication is that the increased salivary secretion observed was due to enhanced water permeability in the normally water-impermeable duct cells.<sup>72</sup> Currently, a phase I study is underway in individuals with salivary hypofunction to see whether hAQP1 gene transfer is safe and effective in humans (<http://www.clinicaltrials.gov/ct/show/NCT00372320>).

### Stem cell therapy

Following irradiation, salivary gland recovery is dependent on the radiation dose and on the number of remaining viable stem cells.<sup>47</sup> These findings indicate that similar to other organs, in salivary glands, lack of replenishment of functional cells is due to the radiation-induced loss of the tissues' endogenous gland stem cells.<sup>13</sup> Increasing the regenerative potential of salivary glands by stem cell therapy after irradiation should be able to restore tissue homeostasis.

Lombaert *et al.*<sup>73</sup> recently discovered a population of c-Kit<sup>+</sup> cells with the capability to restore radiation-induced damage to salivary glands in rodents. Stem cell-containing salispheres were cultured from rodent submandibular glands. *In vitro*, cells from these spheres expressed many stem cell markers (e.g., Sca-1, c-Kit, Musashi-1) and were able to differentiate into all salivary gland lineages.<sup>73</sup> Following stem cell enrichment, c-Kit<sup>+</sup> cells were able to regenerate and completely restore submandibular gland function in irradiated secondary recipients three months after transplantation,<sup>73</sup> indicative of two essential characteristics of stem cells, the capability to self-renew and to differentiate into all lineages of an organ (Fig. 4). Importantly, salispheres grown from human parotid and submandibular salivary glands also contained c-Kit<sup>+</sup> cells and showed self-renewal and differentiation capacities *in vitro*.<sup>73</sup> bringing human clinical application of such therapy within reach.

### Epilogue

Despite advances in our understanding of the cellular and biochemical basis for irradiation-induced loss of salivary gland function, options for the clinical management of irradiation-induced salivary gland hypofunction remain largely limited to palliative therapies. Efforts to protect or diminish irradiation-induced damage including IMRT and the use of radioprotectors such as Tempol are progressing; however there is a need for the concurrent pursuit of therapies aimed at restoration of the function of damaged glands. Although most cells in the salivary gland are thought to be post-mitotic, recent studies suggest that an important target of radiation-induced damage may be the relatively small population of salivary stem cells. Identification of this cell population and strategies for the expansion of salivary stem cells for gland regeneration are important goals that may lead to restoration of the functional capacity of damaged glands. Likewise exciting studies are underway to restore the functional capacity of the gland through gene transfer, e.g., hAQP-1, which can enhance fluid formation in damaged salivary gland. This pursuit of these multiple complementary strategies is important if improvements in the clinical management of the oral complications of irradiation are to advance.

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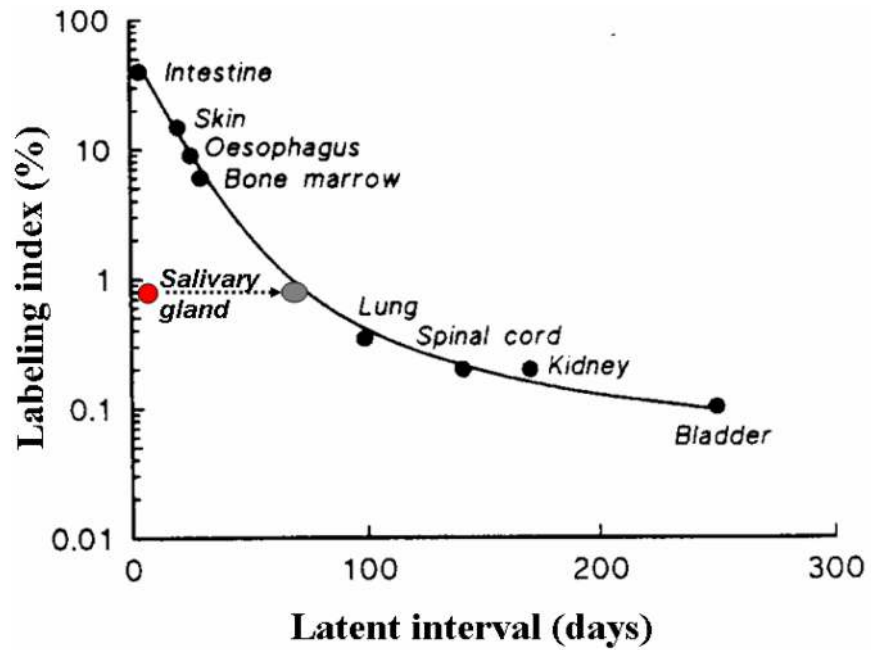
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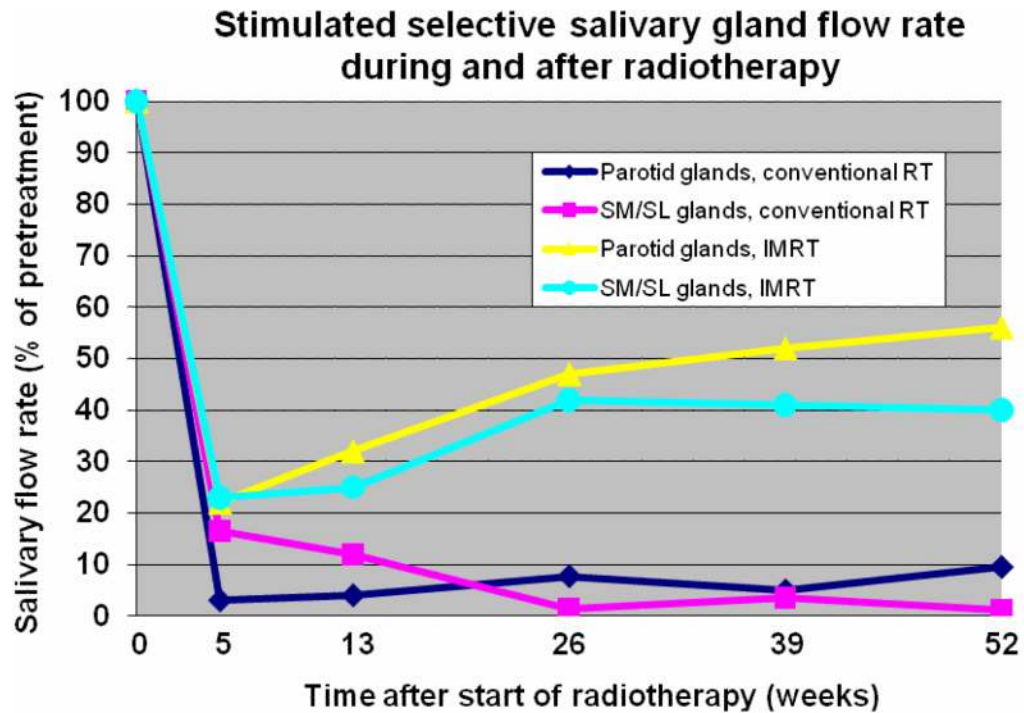


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**Fig. 1.** Based on the slow turnover rates of their cells, salivary glands are expected to be late responding, but the changes in quantity and composition of saliva already occur shortly after radiotherapy (red circle). This resembles an immediacy of a radiation response (short latent interval) that is normally observed for cells with a higher labelling index (such as intestine). However, when one looks at the actual kill of acinar cells, the curve behaves just like any other late responding tissue (gray circle) (adapted from Stewart and van de Kogel, 2002).<sup>7</sup>



**Fig. 2.**

Flow rate of 2% citric acid-stimulated parotid (single gland) and bilateral submandibular-sublingual (SM/SL) saliva as a function of time after start of radiotherapy (RT) [Conventional RT; both parotid, submandibular and sublingual glands located in the treatment portal, 2 Gy per day, 5 days per week, total dose 60–70 Gy. Parotid-sparing 3-dimensional (3D)/intensity-modulated RT (IMRT); bilateral (the majority) and unilateral RT (scattered radiation to contralateral gland). For parotid IMRT data: 1.8–2.0 Gy per fraction, prescribed dose to primary target 64 Gy (range 57.6–72 Gy) and for SM/SL IMRT data: 2 Gy per day, 70 Gy to gross disease planning target volume]. Initial flow rates are set to 100% (modified after Burlage et al.<sup>1</sup> for conventional RT, Eisbruch et al.<sup>22</sup> for parotid glands IMRT, and Murdoch-Kinch et al.<sup>25</sup> for SM/SL glands IMRT).

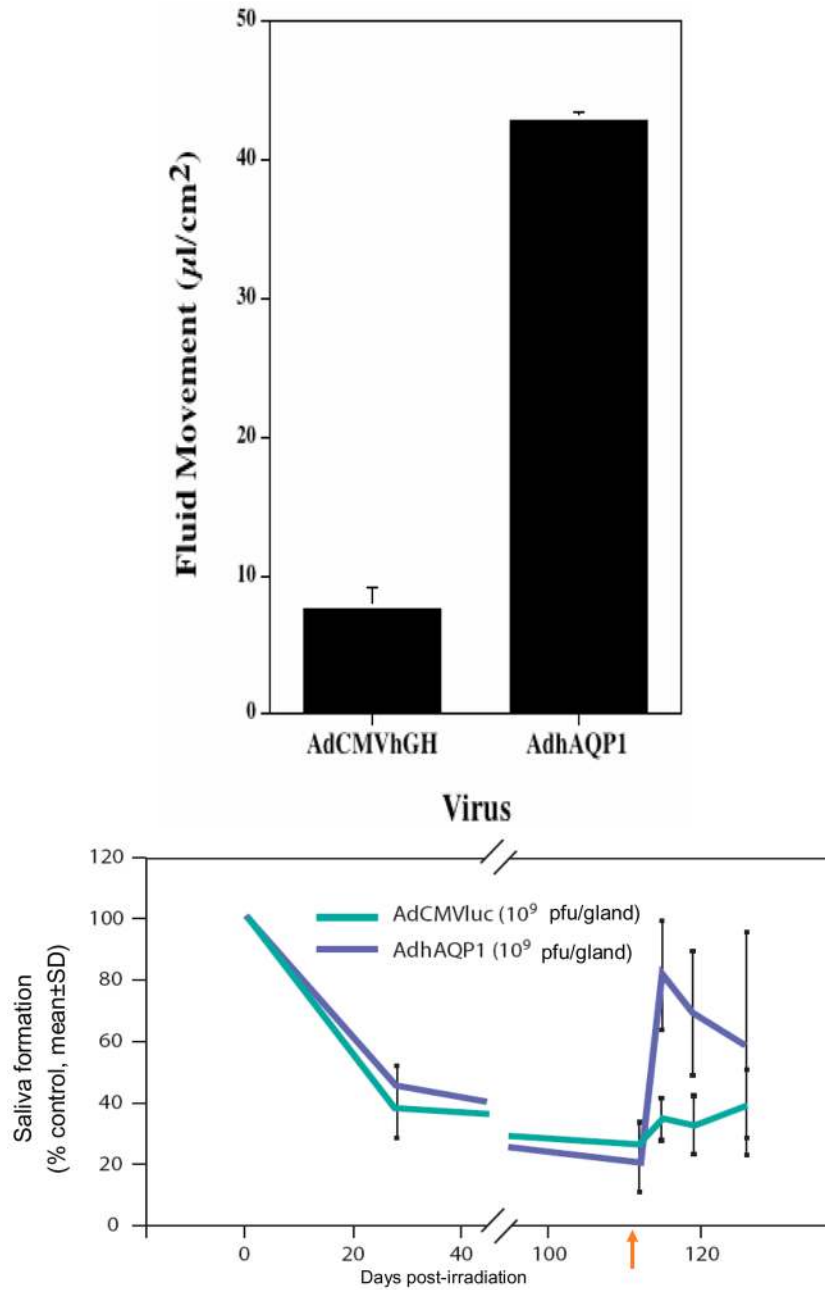
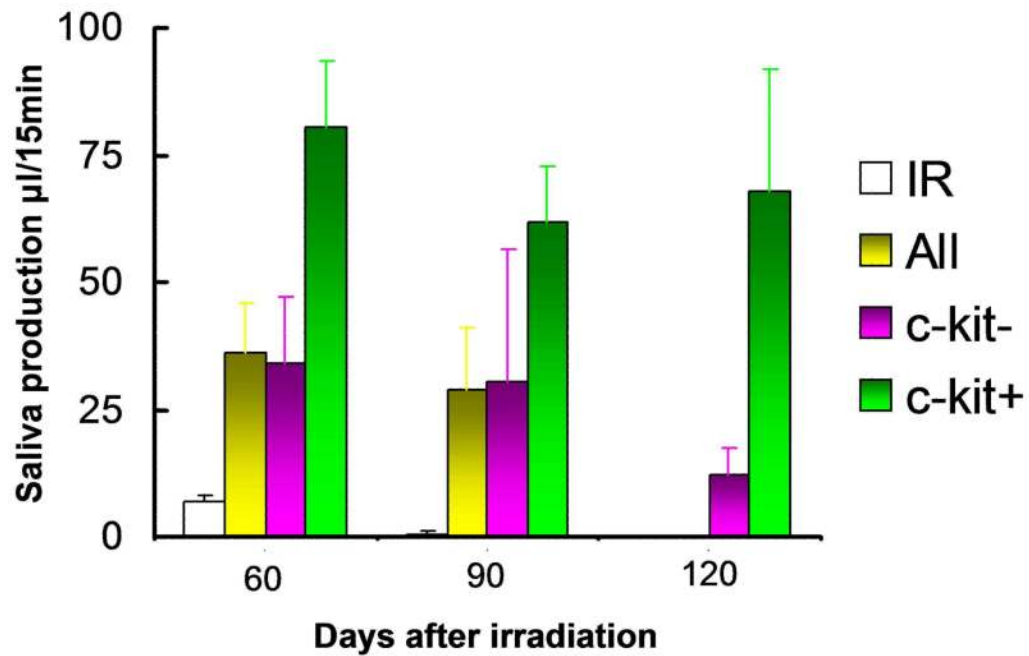
**Fig. 3.**

Fig. 3a Effect of AdhAQP1 on net fluid movement across SMIE cell monolayers. Data shown are the mean  $\pm$  SEM of experiments originally reported in He et al.<sup>73</sup> SMIE cell monolayers were either transduced at a multiplicity of infection of 5 with AdhAQP1 or AdCMVhGH (control vector, encoding human growth hormone), and 24 hours later transepithelial fluid movement was measured for 60 min.<sup>73</sup>

Fig. 3b Parotid salivary output after delivery of an adenoviral vector containing a water channel or control. The flow rate prior to irradiation was set at 100% and the salivary flow rates obtained at times thereafter are represented as a percentage of this initial value. The arrow indicates the time point when the adenoviral vectors were administered. AdhAQP1 is



the vector encoding the water channel transgene hAQP1. AdCMVluc is a control vector (modified after Shan *et al.*, 2005<sup>74</sup>).



**Fig. 4.** Restored organ function by transplanted progenitor/stem cells. Cells of day 3 old salispheres cultured from dispersed submandibular gland cells were intra-glandularly injected 30 days post-irradiation. Injection of 300–1000 c-Kit<sup>+</sup> cells (green bars) restored gland function up to 120 days post-irradiation, whereas 10,000–90,000 c-Kit<sup>-</sup> only temporarily, and to a much lesser extent, restored function (up to 90 days) indicating that they may also contain some progenitor cells. All (red) all cells of the salispheres; (n.d. not determined).

**Table 1**

Guidelines for a variety of management strategies aimed to reduce xerostomia and their level of evidence (for details see the systematic review by Jensen *et al.*<sup>6</sup>). Quality of recommendations according to the American Society of Clinical Oncology clinical practice guidelines.<sup>39</sup>

Treatment approach	Guideline	Level of evidence
<i>Radical scavenger</i>		
Amifostine	Phase III trials have shown that amifostine might reduce xerostomia after radiotherapy. However, no guidelines are possible due to lack of consensus on interpretation of existing evidence. Most studies did not have the statistical power to evaluate the influence of amifostine on the therapeutic index. Also the trial design of most studies was at least questionable and the outcomes subject to debate. The majority of the trials lacked a placebo in the control arms.	Level II, grade C
<i>Muscarinic agonist stimulation</i>		
During radiotherapy	Use of pilocarpine can not be recommended for improvement of xerostomia as the results of the randomized clinical trials were not univocal. Moreover, improvement of salivary gland hypofunction was limited. The dissimilar results on sparing of the salivary gland function are probably highly dependent on the wide range of cumulative doses applied. The only trial providing an analysis of sparing of parotid gland function related to mean parotid dose indicated significant sparing of parotid gland function and reduced xerostomia for mean parotid doses exceeding 40 Gy. <sup>38</sup>	Level II, grade C
After radiotherapy	The use of pilocarpine after radiotherapy can be recommended for improvement of xerostomia	Level II, grade B
<i>Mucosal lubricants/saliva substitutes</i>	The use of oral mucosal lubricants and saliva substitutes for short-term improvement of xerostomia after radiotherapy can be recommended.	Level II, grade B
<i>Acupuncture</i>	The use of acupuncture to stimulate salivary gland secretion and to alleviate xerostomia can be suggested to the patient.	Level II, grade C

**Table 2**

## Sialogogues.

<b>Gustatory and tactile sialogogues*</b>	<b>Pharmacological sialogogues</b>
Acid-tasting substances	Anetholetrithione
acidic (sugar-free) sweets	Benzapyrone
acidic or effervescent drinks (lemon juice, citric acid, buttermilk)	Betanechol chloride
	Carbachol
citric acid crystals	Cevimeline**
cotton-wool gauze soaked in a citric acid and glycerine solution	Folia Jaborandi and tinctura Jaborandi
	Neostigmine, neostigmine bromide, pyridostigmine bromide, destigmine bromide
lemon pastilles	
lemon slices	Nicotinamide and nicotine acid
vitamin C tablets	Pilocarpine hydrochloride**, pilocarpine nitrate
Miscellaneous substances:	Potassium iodide
dried pieces of reed root (calami rhizome)	Trithioparamethoxyphenylpropene
sugar-free chewing gum	
sugar-free sweets	
vegetables or fruits	

\* Not all substances are recommended in dentulous patients as acidic products may induce demineralization of dental hard tissues

\*\* Only these agents have been approved for relief of xerostomia in humans and have undergone substantial, controlled clinical testing

**Table 3**Recommendations for the treatment of hyposalivation with oral mucosa lubricants and saliva substitutes.<sup>66</sup>

<i>Severe hyposalivation</i>	A saliva substitute with gel-like properties should be used during the night and when daily activities are at a low level. During the day, a saliva substitute with properties resembling the viscoelasticity of natural saliva, such as substitutes which have xanthan gum and mucin (particularly bovine submandibular mucin) as a base should be applied.
<i>Moderate hyposalivation</i>	If gustatory, tactile or pharmacological stimulation of the residual salivary secretion does not provide sufficient amelioration, saliva substitutes with a rather low viscoelasticity, such as substitutes which have carboxymethylcellulose, hydroxypropylmethylcellulose, mucin (porcine gastric mucin), or low concentrations of xanthan gum as a base are indicated. During the night or other periods of severe oral dryness, the application of a gel is helpful.
<i>Slight hyposalivation</i>	Gustatory, tactile or pharmacological stimulation of the residual secretion is the treatment of choice. Little amelioration is to be expected from the use of saliva substitutes.