

## Clinical Investigation

# Results of a Phase 1 Open-Label, Dose-Escalation Study of Gene Therapy With AAV2-hAQP1 for Grade 2 and 3 Radiation-Induced Late Xerostomia and Parotid Gland Hypofunction

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**Purpose:** Grade 2 and 3 late xerostomia is a chronic, debilitating complication of radiation therapy for head and neck cancers. We assessed the safety and efficacy of AAV2-hAQP1 gene therapy as a treatment for this condition.

**Methods and Materials:** Twenty-four participants who reported grade 2 and 3 late xerostomia at screening,  $\geq 5$  years after completing their final radiation therapy treatment ( $\geq 2$  years if human papillomavirus-positive), were enrolled in this open-label, multicenter, dose-escalation study. AAV2-hAQP1 was delivered to the parotid gland(s) via cannulation of Stensen's duct. Twelve participants received AAV2-hAQP1 in 1 gland and 12 in both glands. Participants were followed for 12 months. Safety parameters included adverse events, physical examinations, laboratory tests, and electrocardiograms. Efficacy assessments included the Xerostomia-specific Questionnaire (XQ), MD Anderson Symptom Inventory-Head and Neck Module (MDASI-HN), Global Rate of Change Questionnaire (GRCQ), and measurement of unstimulated whole saliva flow rates (UWSFR).

**Results:** No treatment-related serious adverse events or dose-limiting toxicities were reported, and all participants completed the study. When the data from all cohorts were pooled, statistically significant improvements were seen on all patient-reported outcomes by day 30. These were maintained through month 12 with greater improvement in the bilateral cohort. At month 12, the mean percent change from baseline (%CFB) was  $-39.5\%$  and  $-42.2\%$  for the XQ Total Score and the MD Anderson Symptom Inventory-Head and Neck Module dry mouth question, respectively, and the mean GRCQ-Symptom Change Score was 3.8. Overall, 16 of 24 (67%) participants reported an improvement of  $\geq 8$  points in XQ Total Score, and 19 of 24 (79%) participants reported important improvements in xerostomia symptoms based on the GRCQ. The mean %CFB in UWSFR at month 12 was 112.5%.

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**Conclusions:** Treatment with AAV2-hAQP1 was safe and well-tolerated at all doses and resulted in meaningful improvements in xerostomia symptoms and UWSFR. © 2026 Published by Elsevier Inc.

## Introduction

Every year in the United States, ~65,000 people are treated for head and neck cancer, and radiation therapy is a component of the treatment plan for ~75% of those affected.<sup>1</sup> Therapeutic radiation disrupts or destroys the salivary glands' cellular architecture and function. As a result, nearly all patients who receive radiation therapy for head and neck cancer experience some degree of xerostomia, defined as a "patient-reported, subjective sense of oral dryness."<sup>2</sup> Radiation-induced xerostomia (RIX) remains one of the most prevalent and serious side effects of radiation therapy, afflicting tens of thousands of head and neck cancer survivors in the United States every year.

In 2021, the Multinational Association of Supportive Care in Cancer/International Society of Oral Oncology (MASCC/ISOO) and American Society of Clinical Oncology convened a multidisciplinary Expert Panel to evaluate the evidence and formulate recommendations for salivary gland hypofunction and/or xerostomia induced by nonsurgical cancer therapies,<sup>2</sup> issuing the first guidelines for this condition. The panel made efforts to distinguish between xerostomia and salivary gland hypofunction. As noted in the guidelines, "Although xerostomia most frequently occurs when the unstimulated whole saliva flow rate (UWSFR) is reduced by about 45%-50% of the normal secretion of that person, there are no specific threshold levels of salivary flow rate that characterize xerostomia. The degree of xerostomia may be affected by factors other than salivary flow rates."

Within 24 months of completing radiation therapy, nearly half of patients report recovery from xerostomia, but a majority report persistent (late) xerostomia that is lifelong.<sup>3,4</sup> Of patients with late xerostomia, ~80% describe their condition as moderate (grade 2) or severe (grade 3) and experience subjective (eg, burning mouth, dysgeusia, and difficulties with swallowing, speech, and sleep) and objective (eg, oral infections, oral candidiasis, carious destruction of teeth, mucosal erosion, and weight loss) morbidities.

The Guidelines include three treatment recommendations for RIX: (1) oral mucosal lubricants/saliva substitutes; (2) gustatory and masticatory salivary reflex stimulation by lozenges, gums, and candies; and (3) oral sialagogues (pilocarpine and cevimeline). Regarding the last, the Guidelines caution, "However, improvement of salivary gland hypofunction may be limited."<sup>2</sup> Tolerability is also a major issue with sialagogues because of their nonselective nature and resultant off-target parasympathetic effects (eg, low heart rate, low blood pressure, excessive sweating, diarrhea, and other gastrointestinal disturbances).

A U.S. Food and Drug Administration Externally Led, Patient-Focused Drug Development (EL-PFDD) Meeting, convened in August 2021, surveyed 54 patients with active

RIX, and only 15% reported that currently available treatments control their xerostomia "to a great extent," with 85% responding "somewhat" or "very little."<sup>5</sup> Furthermore, when asked about the biggest drawback of their current treatment regimen, 71% reported "not very effective," and 25% reported "side effects." In summary, available treatments provide inadequate relief for most patients and are often poorly tolerated.

Delivery of the human aquaporin-1 (AQP1) gene to parotid glands using AAV2-hAQP1 is designed to address the significant unmet medical need of patients with grade 2 and 3 radiation-induced late xerostomia.<sup>6</sup> AAV2-hAQP1 is introduced directly to the parotid gland epithelium via a retroductal cannulation of Stensen's duct. The vector expresses AQP1, an always-open, archetypal water-specific membrane channel that induces circumferential permeability to water in the epithelial cell membranes. When expressed in acinar and ductal cells of the parotid gland, this allows interstitial fluid to move down its hydrostatic and osmotic gradient into the intraductal lumen, drain into the oral cavity, and wet the mucosal surfaces. This approach was developed in irradiated rodent models before advancing to the irradiated miniswine model, where improvements in salivary flow rates were shown.<sup>6</sup> Subsequently, a first-in-human study using adenovirus type 5 as the vector for delivery of the AQP1 gene to parotid glands of patients with RIX reported improvements in subjective measures of dry mouth and objective measures of parotid saliva flow rate with no notable safety signals.<sup>7</sup>

Adeno-associated virus (AAV)-based vectors have demonstrated lower immunogenicity and more stable expression than adenovirus vectors, and AAV vectors of various serotypes have shown clinical efficacy, resulting in U.S. Food and Drug Administration approval for indications such as RPE65-dependent congenital vision loss,<sup>8</sup> spinal muscular atrophy,<sup>9,10</sup> Duchenne muscular dystrophy,<sup>11</sup> hemophilia A<sup>12</sup> and B,<sup>13</sup> and sickle cell disease.<sup>14,15</sup> Specific to the AAV2 serotype, expression has been long-lasting in human cells and tissues with minimal host immune response following a single treatment,<sup>16</sup> and in explants of human primary parotid gland epithelium, strong ex vivo binding has been demonstrated.<sup>17</sup>

We report data from this 12-month trial of patients with grade 2 and 3 radiation-induced late xerostomia treated with AAV2-hAQP1. Per regulatory requirements, trial participants were asked to enroll in a long-term follow-up study that will continue to evaluate safety and efficacy for 5 years post-treatment.

## Methods and Materials

This open-label, multicenter, dose-escalation trial enrolled males and females aged  $\geq 18$  years with a history of radiation therapy for head and neck cancer (a minimum of 5 years

since completing radiation therapy if human papillomavirus status was negative or unknown and a minimum of 2 years if human papillomavirus-positive). Participants were screened for patient-reported grade 2 and 3 late xerostomia, abnormal parotid gland function (defined as no collectable unstimulated parotid salivary flow and a stimulated parotid salivary flow  $>0$  and  $<0.3$  mL/min/gland after 2% citrate stimulation), and had no evidence of head and neck cancer as determined by an ENT exam and a CT scan of the head, neck, and chest with contrast. Institutional review board approval and informed consent were obtained before any study-related assessments were performed. Before study drug administration, patient self-reported outcome (PRO) questionnaires were administered, and salivary flow rates were measured to establish a baseline. Dosing was carried out via cannulation of Stensen's duct after intravenous injection of glycopyrrolate to block saliva secretion, as previously reported.<sup>7</sup> The doses administered were based on earlier preclinical research in the miniswine model of RIX.<sup>18</sup>

The primary endpoint was the safety of AAV2-hAQP1 administered to one or both parotid glands of adult participants with RIX. Safety parameters included assessments of adverse events, vital signs, physical examination findings, clinical laboratory results, and electrocardiogram (ECG) findings. The effectiveness of AAV2-hAQP1 was evaluated via assessments of: (1) xerostomia symptoms using the Xerostomia-specific Questionnaire (XQ), the MD Anderson Symptom Inventory - Head

and Neck Module (MDASI-HN), and the Global Rate of Change Questionnaire adapted to xerostomia (GRCQ), and (2) unstimulated and/or stimulated salivary output (mL/min) as compared with baseline.

The trial began with unilateral dosing at the doses listed in Table 1, with bilateral dosing added in a protocol amendment. Each dose-escalation cohort enrolled 3 participants. Vector doses are presented as vector genomes (vg)/mL. These vector concentrations were obtained by PCR methodology performed on the drug product as part of a battery of quality control testing before release.

Dose-limiting toxicity (DLT) was defined as any of the following:

- (1) CTCAE grade  $\geq 3$  treatment-emergent adverse event (TEAE) possibly or probably related to AAV2-hAQP1, or
- (2) CTCAE grade  $\geq 3$  administration site reaction not responsive to anti-inflammatory treatment with prednisone.

For each of the unilateral cohorts, a single initial participant was dosed and observed for 15 days. When no DLT was observed, the remaining 2 participants in that cohort were dosed. The decision to advance to the next dose-escalation cohort was made after the final participant in the current cohort was observed for 30 days post-dosing. Once dosing in unilateral cohort 3 ( $1 \times 10^{12}$  vg/gland) was well-tolerated,

**Table 1 Participant demographics**

Cohort dose level (vg/mL) (cohort size)	Cohort 1 $1 \times 10^{11}$ (n = 3)	Cohort 2 $3 \times 10^{11}$ (n = 3)	Cohort 3 $1 \times 10^{12}$ (n = 3)	Cohort 4 $3 \times 10^{12}$ (n = 3)	Cohort 1b $3 \times 10^{10}$ (n = 3)	Cohort 2b $1 \times 10^{11}$ (n = 3)	Cohort 3b $3 \times 10^{11}$ (n = 3)	Cohort 4b $1 \times 10^{12}$ (n = 3)	All subjects (n = 24)
Age (y)									
n	3	3	3	3	3	3	3	3	24
Mean (SD)	71.3 (7.51)	57.3 (7.51)	63.0 (1.73)	68.0 (8.89)	66.7 (2.52)	50.7 (2.52)	65.0 (9.64)	66.3 (2.89)	63.5 (8.16)
Median	71.0	53.0	62.0	71.0	67.0	51.0	61.0	68.0	64.0
Min, Max	64, 79	53, 66	62, 65	58, 75	64, 69	48, 53	58, 76	63, 68	48, 79
Sex, n (%)									
Male	3 (100.0)	3 (100.0)	3 (100.0)	2 (66.7)	2 (66.7)	2 (66.7)	2 (66.7)	3 (100.0)	20 (83.3)
Female	0	0	0	1 (33.3)	1 (33.3)	1 (33.3)	1 (33.3)	0	4 (16.7)
Race, n (%)									
Black or African American	1 (33.3)	0	0	0	0	0	0	0	1 (4.2)
White	2 (66.7)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	23 (95.8)
BMI (kg/m <sup>2</sup> )									
n	3	3	3	3	3	3	2	0	20
Mean (SD)	28.3 (5.67)	25.2 (3.37)	25.1 (1.11)	26.2 (6.77)	31.5 (10.69)	26.7 (1.82)	25.6 (7.75)	NA (NA)	27.0 (5.48)
Median	31.3	23.6	25.5	28.7	35.2	27.7	25.6	NA	26.8
Min, Max	21.7, 31.8	22.9, 29.1	23.8, 25.9	18.6, 31.4	19.5, 39.9	24.6, 27.8	20.1, 31.1	NA, NA	18.6, 39.9

dosing began in bilateral cohort 1b ( $3 \times 10^{10}$  vg/gland), and dosing proceeded in all bilateral cohorts without pauses.

After dosing, participants returned for follow-up visits on days 2, 8, 15, 30, 60, 90, 180, and 360. Safety assessments included adverse events (AEs), vital signs, oral exams, clinical laboratory tests at all visits, and ECGs (at screening, day 30, and day 360). Over-the-counter dry mouth remedies were prohibited for 2 hours before clinic visits, and prescription sialagogues were prohibited 24 hours before all clinic visits. At each visit (including both screening and postdosing periods), participants were asked to complete 3 questionnaires: (1) the Xerostomia-specific Questionnaire (XQ)<sup>19</sup>, (2) MDASI-HN<sup>20,21</sup>, and (3) the GRCQ<sup>22</sup> (Appendix E1). The following PROs were obtained: (1) XQ Total Score (sum of 8 individual item scores, range, 0-80); (2) MDASI-HN domain scores (range, 0-10); (3) GRCQ-Symptom Change Score (GRCQ-S), and GRCQ Functional Limitation Change Score (GRCQ-FL) (range 1-7). In addition, the UWSFR (mL/min) was determined by asking the participant to sit comfortably with their head inclined slightly forward and passively spit saliva into a collection tube for 3 to 5 minutes.

Responder analyses using the XQ and GRCQ data were performed using the following definitions:

Responder:

- XQ: a  $\geq 8$ -point decrease in XQ Total Score at the month 12 visit, compared with baseline, or
- GRCQ: a  $\geq 2$ -point change in either GRCQ-S or GRCQ-FL at the month 12 visit.

The Responder threshold for the XQ is based on earlier work suggesting that a CFB of 8 points may represent the minimal clinically important difference in the scale.<sup>23</sup> The Responder threshold for the GRCQ is based on the wording of the questionnaire, wherein participants describe a change of  $\geq 2$  as an “important” improvement.

Only question 10 in the MDASI-HN module directly addresses dry mouth and is referred to herein as the “Dry Mouth” domain, or MDASI-DM. Therefore, the analysis of MDASI-HN focuses on the responses to this question. Baseline values were derived by averaging data collected at visits during the screening period and before study drug administration (3 times for PROs and twice for saliva flow rate). The efficacy data were analyzed using a mixed model for repeated measures, with visit as the fixed effect, baseline value and actual dose concentration as covariates, and the individual participant as the random effect. As specified in the Statistical Analysis Plan, key elements of which are provided in the Appendix E1, statistical analyses were descriptive, and no formal hypothesis testing or comparative analyses between cohorts were performed.

The trial was conducted in compliance with the approved protocol, the International Council for Harmonization Good Clinical Practice guidelines, the Declaration of Helsinki, and all applicable federal, state, and local laws, rules

and regulations relating to the conduct of clinical trials. In addition, the protocol, any amendments, and the informed consent form were approved by the appropriate institutional review board before implementation.

## Results

### Study population

Twenty-four participants were enrolled in the study (ie, comprised the full analysis population) at 5 sites in North America. All participants received the study drug (4 cohorts of 3 participants each received unilateral treatment, and 4 cohorts of 3 participants each received bilateral treatment) and were included in the Safety Population. All 24 participants completed the study per protocol.

Most participants were male (83.3%) and White (95.8%). Age ranged between 48 and 79 years (mean 63.5 years), and the mean body mass index was 27.0 kg/m<sup>2</sup>. Participant demographics are presented in Table 1 by the dose received. The collection of mean parotid radiation therapy doses was not specified in the study protocol. These data were able to be retrieved for 16 of the 24 study participants and are included in Table 2 along with participant radiation therapy and chemotherapy treatment plans.

### Safety

Treatment-related treatment-emergent adverse events (TEAE)s were reported for 6 of 24 participants (25.0%). All were mild (CTCAE grade 1) in intensity and resolved without sequelae. Table 3 summarizes the TEAEs assessed by the investigator as related to the study drug. No TEAE resulted in study discontinuation.

Serious Adverse Events (SAEs) were reported for 2 participants (8.3%). One participant in cohort 3 was hospitalized with grade 3 obstructive airways disorder on day 360, which resolved the following day. One participant in cohort 4 was hospitalized with grade 3 coronary artery disease on day 209, which also resolved the following day. Neither of the SAEs was assessed by the investigator as related to study treatment.

No participants had a DLT. The investigator assessed 8 TEAEs in 6 participants as probably or possibly related to study treatment and grade 1 in intensity. Of these 8 TEAEs, 6 resolved within 24 hours, whereas Increased Serum Amylase persisted for 7 days, and oral disorder (reported as a reticular lichenoid pattern on the oral mucosa) was present at the day 7 visit and had resolved by the day 90 visit. TEAEs of special interest (defined as: parotid gland tenderness, swelling, pain, or rubor; blood in parotid saliva; or adenopathy in the lymph nodes draining the parotid gland) were reported for 3 participants (12.5%). Of these, oral disorder and injection site pain were assessed by the

**Table 2 Participant cancer treatment plans**

Cohort	Participant ID	Location	Chemotherapy	Radiation type	Total dose	Mean parotid radiation therapy dose (L/R)*	Years post-RT at enrollment
1	S001	Neck	None	IMRT	7000 cGy	N/A	11.84
1	S002	Tonsil	Cetuximab	IMRT	7000 cGy	3715 cGy/2595 cGy	6.69
1	S003	Left neck, left tonsil, and base of tongue; 5400cGy to right neck	Carboplatin	IMRT	6000 cGy	N/A	10.99
2	S004	Tongue	Cisplatin	IMRT	7020 cGy	N/A	6.32
2	S005	Tongue	Cisplatin	IMRT	7000 cGy	3360 cGy/2586 cGy	2.95
2	S006	Base of tongue and lower anterior neck	Cisplatin	IMRT	7020 cGy	N/A	7.02
3	S007	Neck and left tonsil	Cisplatin	IMRT	7000 cGy	N/A	5.86
3	S008	Oropharynx	None	IMRT	7000 cGy	6016 cGy/2580 cGy	7.23
3	S009	Tongue	Cisplatin	IMRT	7000 cGy	2586.7 cGy/5296.8 cGy	4.38
4	S010	Oropharynx	Cisplatin	IMRT	7000 cGy	4264.3 cGy/2302.6 cGy	7.51
4	S011	Tonsil	Cisplatin	IMRT	7000 cGy	7020 cGy/5240 cGy	16.03
4	S012	Tongue	Cisplatin	IMRT	7000 cGy	2477.2 cGy/2301.2 cGy	3.04
1b	S013	Tonsil	Cisplatin	IMRT	6996 cGy	3071 cGy/3604 cGy	3.97
1b	S014	Tonsil	Cisplatin	IMRT	7000 cGy	2475.5 cGy/4609.6 cGy	5.22
1b	S015	Tonsil	Cisplatin	IMRT	7000 cGy	2125 cGy/4626.3 cGy	3.98
2b	S016	Tonsil and neck	Cisplatin	IMRT	6600 cGy	N/A	6.45
2b	S017	Tonsil	Cisplatin	IMRT	7000 cGy	2534.3 cGy/2902.7 cGy	4.98
2b	S018	Tongue	Cisplatin	IMRT	7000 cGy	2716.2 cGy/4671 cGy	3.03
3b	S019	Tongue	Cisplatin	IMRT	7000 cGy	2523.4 cGy/4671 cGy	3.45
3b	S020	Tongue	None	IMRT/ concomitant boost	5800 cGy/7200 cGy	N/A	13.97
3b	S021	Tongue	None	IMRT	5800 cGy	N/A	3.56
4b	S022	Oropharynx	Cisplatin	IMRT	7000 cGy	5640 cGy/2000 cGy	6.19
4b	S023	Oropharynx	Carboplatin/ induction docetaxel, cisplatin, and fluorouracil	Unknown	7000 cGy	1820 cGy/5720 cGy	8.1
4b	S024	Tongue	None	IMRT	7000 cGy	2800 cGy/4300 cGy	6.85

*Abbreviations:* IMRT = intensity modulated radiation therapy; N/A = not available.  
\*Mean parotid radiation therapy doses were collected post hoc and were not independently verified. Accordingly, analyses thereof were not performed.

investigator as related to the study treatment, and salivary gland pain was assessed as not related to the study treatment. All TEAEs of special interest were assessed as grade 1 in intensity.

No safety signals emerged from a review of vital signs parameters, oral or physical examinations, laboratory parameters, or ECG interpretation.

### **Efficacy**

Improvements in symptoms of xerostomia and UWSFR were observed across all dose levels and cohorts, and no clear dose-response relationship was detected. As a result, proof-of-concept efficacy was assessed by pooling data from all unilateral and bilateral participants and comparing them

**Table 3 Treatment-emergent adverse events related to study treatment by system organ class and preferred term**

	Cohort 1 1 × 10 <sup>11</sup> (n = 3) n (%)	Cohort 2 3 × 10 <sup>11</sup> (n = 3) n (%)	Cohort 3 1 × 10 <sup>12</sup> (n = 3) n (%)	Cohort 4 3 × 10 <sup>12</sup> (n = 3) n (%)	Cohort 1b 3 × 10 <sup>10</sup> (n = 3) n (%)	Cohort 2b 1 × 10 <sup>11</sup> (n = 3) n (%)	Cohort 3b 3 × 10 <sup>11</sup> (n = 3) n (%)	Cohort 4b 1 × 10 <sup>12</sup> (n = 3) n (%)	All Subjects (n = 24) n (%)
Total number of TEAEs related to study treatment	1	1	0	3	2	0	1	0	8
Subjects with ≥1 TEAE related to study treatment	1 (33.3)	1 (33.3)	0	2 (66.7)	1 (33.3)	0	1 (33.3)	0	6 (25.0)
System organ class									
Preferred term									
Gastrointestinal disorders	0	1 (33.3)	0	0	0	0	1 (33.3)	0	2 (8.3)
Oral disorder	0	1 (33.3)	0	0	0	0	0	0	1 (4.2)
Salivary gland pain	0	0	0	0	0	0	1 (33.3)	0	1 (4.2)
General disorders and administration site conditions	0	0	0	1 (33.3)	1 (33.3)	0	0	0	2 (8.3)
Chills	0	0	0	0	1 (33.3)	0	0	0	1 (4.2)
Fatigue	0	0	0	0	1 (33.3)	0	0	0	1 (4.2)
Injection site pain	0	0	0	1 (33.3)	0	0	0	0	1 (4.2)
Eye disorders	0	0	0	1 (33.3)	0	0	0	0	1 (4.2)
Eye disorder	0	0	0	1 (33.3)	0	0	0	0	1 (4.2)
Investigations	1 (33.3)	0	0	0	0	0	0	0	1 (4.2)
Amylase increased	1 (33.3)	0	0	0	0	0	0	0	1 (4.2)
Nervous system disorders	0	0	0	1 (33.3)	0	0	0	0	1 (4.2)
Dysgeusia	0	0	0	1 (33.3)	0	0	0	0	1 (4.2)

Abbreviation: TEAE = treatment-emergent adverse event.

with the pretreatment baseline. All efficacy analyses were prespecified and documented in the Statistical Analysis Plan before database lock.

### Patient-reported outcomes

For each participant, the XQ and MDASI-DM were to be completed 11 times, and the GRCQ was to be completed 6 times. The XQ Total Score data were available for 260 of the expected 264 values, whereas the MDASI-DM score data were available for 262 of the expected 264 values. GRCQ data were available for 141 of the expected 144 values.

At baseline, the mean (SD) XQ Total Score was 46.7 (13.85) (ie, 47.9 [14.37] for the unilateral treatment group and 45.6 [13.86] for the bilateral treatment group). The mean XQ Total Score decreased (indicating improvement) during the study. On day 30, the percent change from baseline (%CFB) was -24.6%, and this decrease was maintained through month 12 (range, -42.6% to -37.9%). Sixteen (66.7%) participants met the definition of Responder at month 12.

At baseline, the mean (SD) MDASI-DM score was 7.2 (2.27), 8.0 (1.71) for the unilateral treatment subgroup, and 6.4 (2.57) for the bilateral treatment subgroup. The mean MDASI-DM score decreased (indicating improvement) during the study. On day 30, the %CFB was -29.5%, and this decrease was maintained through month 12 (range, -42.6% to -40.7%). Although the MDASI-DM score is the focus of the efficacy evaluation, the symptom severity and interference subscale scores of MDASI-HN provide a comprehensive assessment of the impact of cancer treatment on patients. Over the 12-month study period, the improvement in these scores was similar to that of the MDASI-DM score (Table 4).

At day 30, the mean (SD) GRCQ-S and GRCQ-FL were 2.3 (1.57) and 0.6 (1.41), respectively. The mean GRCQ-S and GRCQ-FL increased (indicating further improvement) during the study and were 3.8 (2.57) and 2.8 (2.83), respectively, at month 12. Nineteen (79.2%) participants met the definition of Responder at month 12.

Least squares means and the associated 95% confidence intervals (95% CI) are plotted in Figure 1A-D for the CFB

**Table 4 Mean changes from baseline in MDASI-HN subscale scores**

		Baseline	Day 30	Day 60	Day 90	Day 180	Month 12
	Mean subscale scores	3.48	2.18	2.15	1.97	2.01	2.02
Total symptom severity	Change from baseline		-1.3	-1.33	-1.51	-1.47	-1.46
	Change from baseline (%)		-37.5	-38.3	-43.5	-42.4	-41.9
	Mean subscale scores	4.12	2.64	2.63	2.3	2.43	2.53
HN module symptom severity	Change from baseline		-1.48	-1.49	-1.82	-1.69	-1.59
	Change from baseline (%)		-35.8	-36.1	-44.1	-41	-38.6
	Mean subscale scores	3.74	2.29	2.24	2.19	2.14	2.09
Total symptom severity	Change from baseline		-1.45	-1.5	-1.55	-1.6	-1.65
	Change from baseline (%)		-38.9	-40.1	-41.3	-42.8	-44
	Mean subscale scores	2.87	1.2	1.56	1.34	1.46	2.12
Symptom interference	Change from baseline		-1.67	-1.31	-1.53	-1.41	-0.75
	Change from baseline (%)		-58.3	-45.8	-53.2	-49.1	-26.3

Abbreviation: MDASI-HN = MD Anderson Symptom Inventory-Head and Neck Module.

(or change) of outcomes for the 3 PROs. Statistically significant changes relative to baseline were seen for all PRO outcomes by the day 30 visit and were maintained through the month 12 visit. Greater improvement in PROs, as measured by both CFB and %CFB, was generally observed for the bilateral treatment subgroup when compared with the unilateral treatment subgroup.

Figure 1E shows an overlay of the %CFB for XQ Total Score, GRCQ-S, and MDASI-DM to compare the relative magnitude of changes by visit across the three PROs. The %CFB for XQ Total Score and MDASI-DM was calculated as CFB divided by their respective baseline values, with a negative %CFB indicating improvement. To align GRCQ-S with the other PRO outcomes, the sign was reversed, and the equivalent %CFB was calculated by dividing the GRCQ-S by 8, the maximum range of possible scores ([0-7]). Less than 1% of the expected questionnaire responses were missing, with no apparent pattern.

### Saliva flow rates

Unstimulated whole saliva collection was added to the protocol, and the saliva collection process was refined after most of the participants in the unilateral cohorts had been enrolled. Accordingly, UWSFR measurements were available for 4 unilaterally and 11 bilaterally treated participants. Overall, there was a trend toward improvement in UWSFR at days 180 and 360 (data not shown). However, because of the variability of baseline measures of UWSFR, we elected to correct for inter-participant differences in baseline flow rate by calculating %CFB on a participant-by-participant basis before averaging across the study. This approach ensured that the effect sizes were not weighted toward those who produced larger flows at baseline, potentially diluting out important intraparticipant effect sizes that presented as small absolute changes, and vice versa. The mean UWSFR

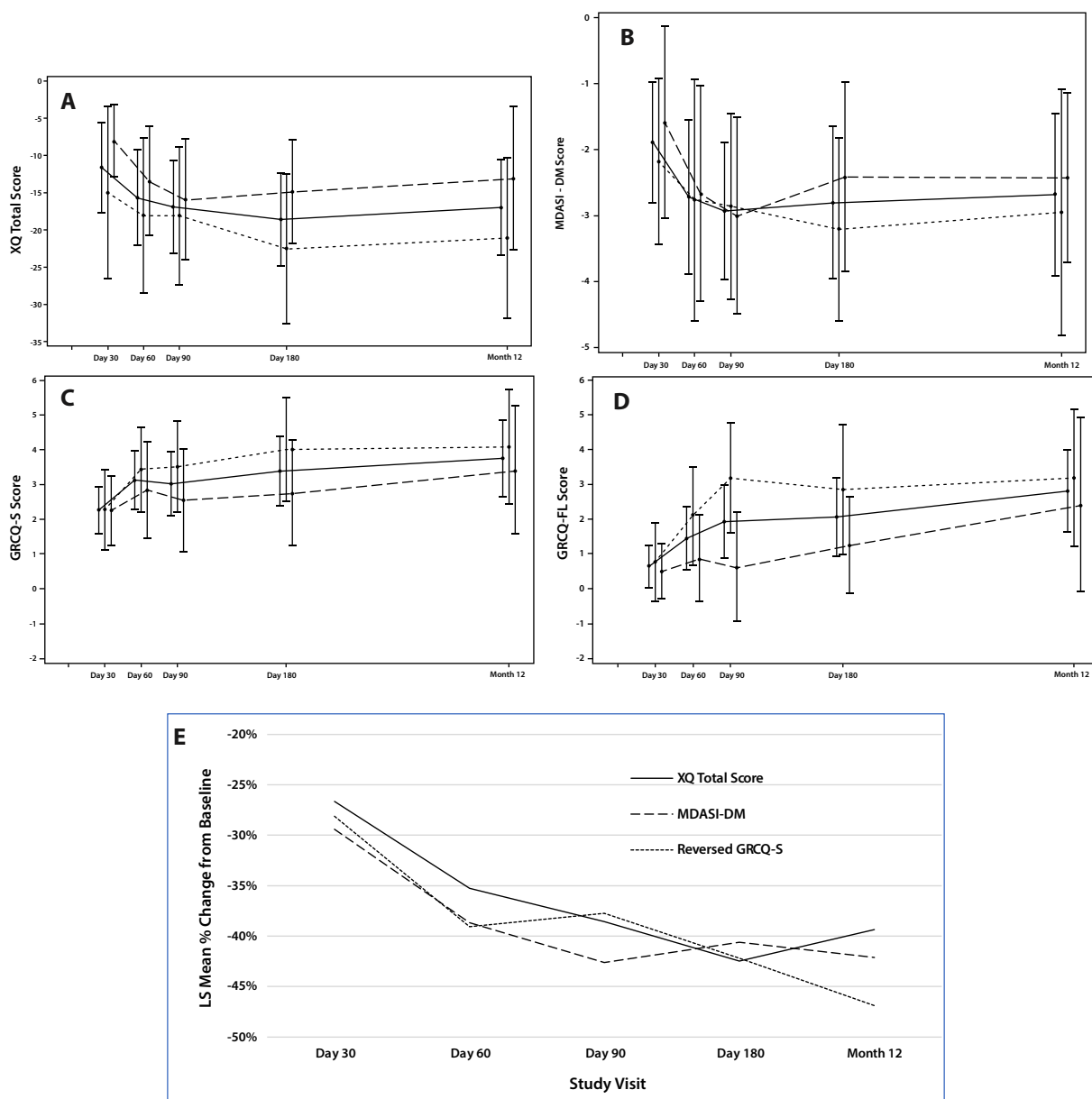
%CFB for this subset of participants generally increased during the study period. On day 30, the mean %CFB in UWSFR was 54.7%, and this increase was maintained through month 12 (range, 56.4%-112.5%; Fig. 2). The median UWSFR increased from 0.15 mL/min at baseline to 0.42 mL/min at month 12.

### Discussion

The symptoms of grade 2 and 3 late xerostomia in head and neck cancer survivors are unremitting and impair quality of life. Current treatment options are few and of limited benefit. In this phase 1 open-label study, AAV2-hAQP1 was well-tolerated at all doses. No treatment-related SAEs or TEAEs led to study discontinuation. TEAEs determined by the investigator to be probably or possibly related to the study treatment were mild in severity and resolved without sequelae.

Participants reported meaningful improvements in xerostomia symptoms as measured by the XQ Total Score, MDASI-DM Score, and GRCQ-Symptom Change Scores. Therapeutic effects were observed within weeks, fully manifested over 2 to 3 months (consistent with observations from other clinical trials using AAV2 vectors), and were durable through the month 12 end-of-study visit. The effect size and response rate with bilateral treatment (dashed lines in Fig. 1A-D) exceeded those of unilateral treatment (dotted lines in Fig. 1A-D). The alignment of results across PROs that measure different aspects of xerostomia symptoms provides compelling proof-of-concept of treatment effectiveness.

As a result of treatment with AAV2-hAQP1, the median UWSFR increased from <0.2 mL/min, the American Society of Clinical Oncology Guidelines definition of salivary gland hypofunction,<sup>2</sup> into the range of normal function, >0.2 mL/

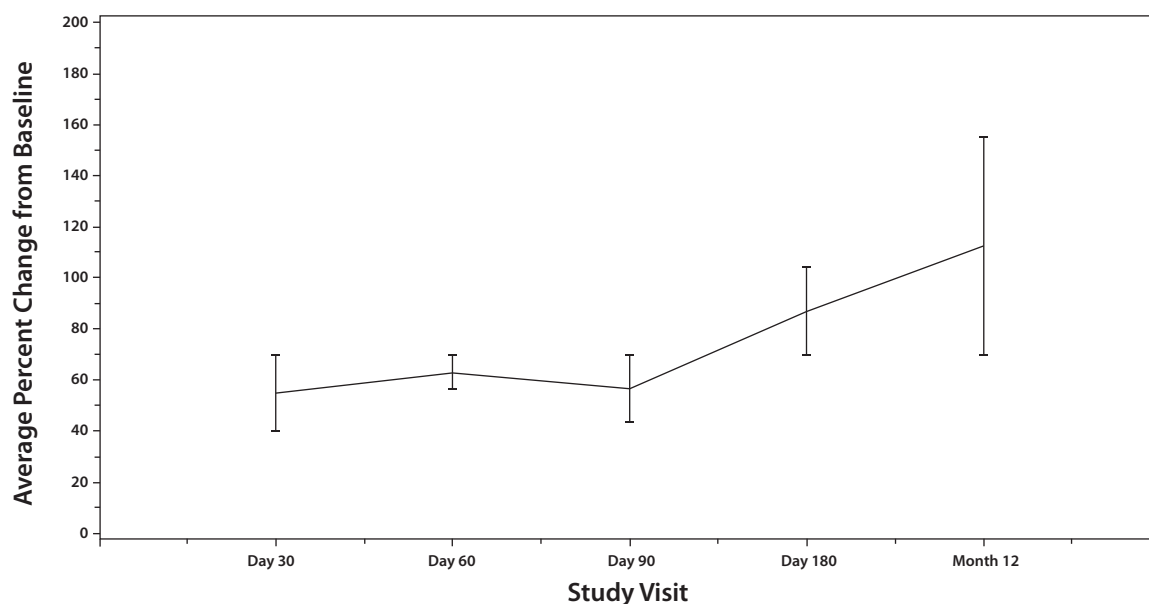


**Fig. 1.** Least squares means (LS-means) of CFB for PRO outcomes for the overall treatment group (solid line), bilateral-treated participants (dotted line), and unilateral-treated participants (dashed line). (A) LS-means (95% CI) for the XQ Total Score Change from baseline, (B) the LS-means (95% CI) for the MDASI-DM Change from baseline, (C) the LS-means (95% CI) for the GRCQ-Symptom Change Score, and (D) the LS-means (95% CI) for the GRCQ Functional Limitation Change Score. For each of the graphs, the x-axis reflects the postdosing visits. (E) LS-means of the Percent Change from Baseline for the XQ Total Score (solid line), MDASI-DM (dashed line), and GRCQ-S (dotted line) by visit. *Abbreviations:* GRCQ = Global Rating of Change Questionnaire; MDASI-DM = the “dry mouth” item 10 from the MD Anderson Symptom Inventory-Head and Neck module; PRO = patient self-reported outcome; XQ = Xerostomia-specific Questionnaire.

min. This enhancement of unstimulated flow is notable because the healthy parotid gland produces primarily stimulated saliva in response to gustatory, visual, or olfactory cues by relocating aquaporin 5 from intracellular vesicles to the cell membrane.<sup>24-27</sup> In contrast, treatment with AAV2-hAQP1 results in unregulated expression of AQP1 on the parotid gland epithelium, rendering it constitutively permeable and enabling a continuous flow of unstimulated saliva

from the interstitium to the parotid duct lumen and into the oral cavity as water flows down the osmotic and hydrostatic gradient.

Xerostomia symptoms and impaired salivary gland function are related but distinct complications of radiation therapy. Xerostomia and UWSFR have been reported not to be strongly correlated,<sup>2</sup> and a similar observation was made in this study (data not shown). However, the concurrent



**Fig. 2.** Unstimulated whole saliva flow rate percent change from baseline by study visit (LS-means and 95% CIs). *Abbreviations:* CI = confidence interval; LS = least squares.

improvements in these measures support the presumed physiological mechanism of action of AAV2-hAQP1 gene therapy.

Efforts were made to collect isolated parotid saliva after stimulation with 2% citric acid swabbing of the tongue, using Lashley cups<sup>28</sup> placed over the orifice of Stensen's duct, per standard methods. The study sites had difficulty in reliably collecting these parotid saliva samples, mostly due to slipped or dislodged collection cups. In total, 344 of 480 (72%) isolated stimulated parotid saliva samples were usable (ie, nonmissing and nonzero). In addition to the technical difficulties experienced, the collection technique used in the study called for measuring only the saliva that drains from the tubing. However, the tubing presents a void volume of 0.5 mL. Calculated flow rates are, therefore, skewed downward, and volumes of <0.5 mL over the collection period would be reported as zero, regardless of whether any saliva was produced. Taken together, these factors dictate that isolated parotid saliva output cannot be reliably measured using the method specified in the study protocol.

Beyond difficulties in accurately measuring the salivary output of individual parotid glands, limitations of this study include a lack of detail in the cancer treatment history (including incomplete detail of mean parotid radiation therapy doses) and the absence of a threshold for xerostomia severity as an entry criterion. The open-label design, although appropriate for a phase 1 trial with a primary endpoint of safety, requires caution in the interpretation of treatment-related improvements in xerostomia symptoms, which are inherently subjective. A phase 2, randomized, double-blind, placebo-controlled trial (NCT05926765) is ongoing, with the change from baseline to month 12 in XQ Total Score and UWSFR as primary and key secondary efficacy endpoints, respectively.

## Conclusions

The study results support the safety, tolerability, and potential therapeutic benefit of AAV2-hAQP1 gene therapy as a one-time, durable, disease-modifying treatment for grade 2 and 3 radiation-induced late xerostomia.

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