

**Open-label, dose-escalation study evaluating the safety of a single administration of an adenoviral vector encoding human aquaporin-1 to one parotid salivary gland in individuals with irradiation-induced parotid salivary hypofunction**

**NIH protocol 06-D-0206; IND 13,102**

**Bruce J. Baum**

**Director, Medical Research Scholars  
Program, NIH Clinical Center**

**Former Chief, Gene Transfer Section,  
NIDCR, NIH**

# Head and Neck Cancers

## Incidence:

~40,000 new cases/yr  
in USA; 500,000/yr  
worldwide



## Treatment:

- Radiation therapy, chemotherapy, surgery
- Oral consequences of radiation therapy include: mucositis, osteoradionecrosis, salivary hypofunction

# Too little saliva leads to considerable morbidity

- Xerostomia (dry mouth)
- Dysphagia (difficulty swallowing)
- Oral infections (Candidiasis, caries)
- Reduced mucosal healing
- Oral pain and discomfort
- Markedly reduced quality of life

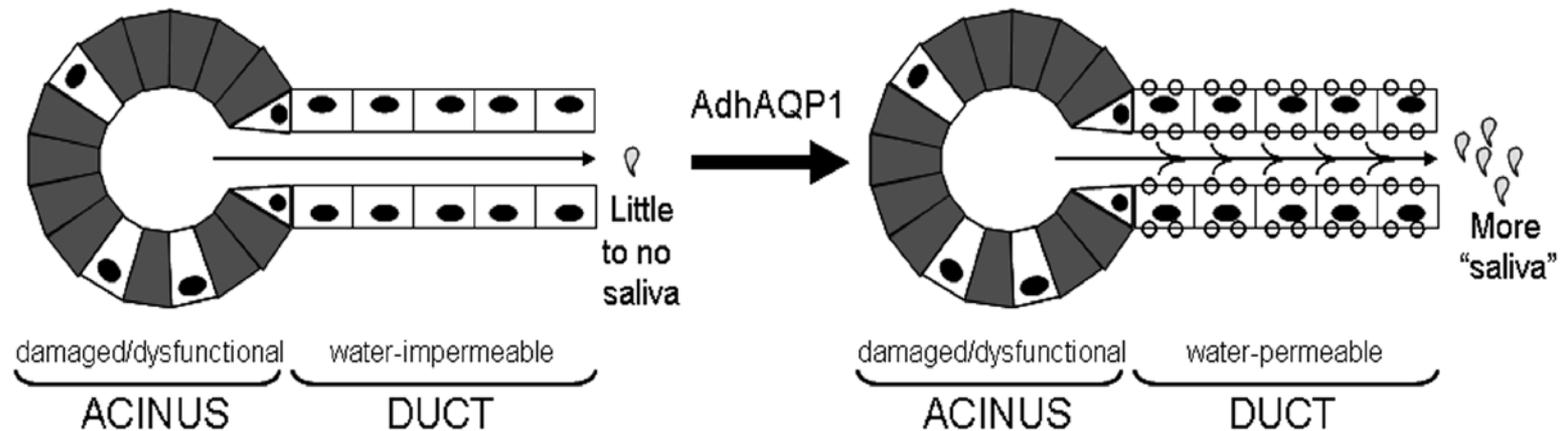
# Radiation Therapy Oncology Group categories of salivary gland dysfunction

Cox et al, IJROBP, 1995

- Grade 0- None
- Grade 1- Slight dryness with good response to stimulation
- Grade 2- Moderate dryness with poor response to stimulation
- Grade 3- Complete dryness with no response to stimulation
- Grade 4- Fibrosis

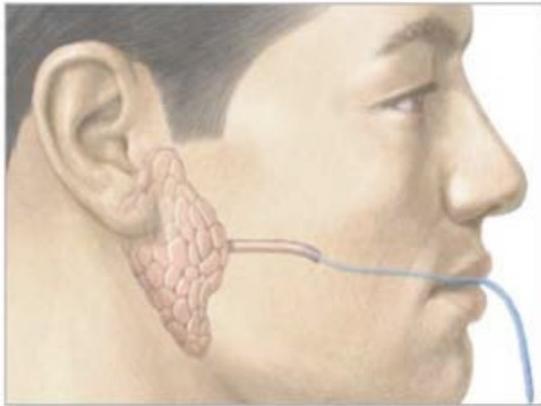
For Grade 2-4 patients there is **no effective therapy** currently available

# General strategy for the “repair of irradiation damage” for RTOG patient grades 2/3



Baum et al, Oral Oncol, 2010 (review)  
Delporte et al, PNAS, 1997 (original data; rat)  
Shan et al, Mol Ther, 2005 (original data; minipig)

## Vectors delivered by intraductal cannulation and retrograde infusion



Contrast medium is injected into the salivary gland duct

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**Sialography is a common clinical procedure**



**Vector delivery to rat salivary glands**



**AdhAQP1 vector delivery to patient in 06-D-0206**

# Phase 1 clinical trial development

- Plan - treat previously irradiated patients in one parotid gland
- Submit clinical protocol (late 2005)
- Required approvals (by early 2007):  
NIDCR-IRB, Recombinant DNA  
Advisory Cmte, NIH Biosafety Cmte,  
Food and Drug Administration, Data  
Safety & Monitoring Board
- Develop infrastructure (2007-2008)
- First patient treated (summer 2008)

# Objectives

- To evaluate the safety of single escalating doses of AdhAQP1
- To evaluate the effectiveness of AdhAQP1 in increasing parotid gland salivary output and reducing complaints of xerostomia

# Study Design

- Open label, single center, single dose, dose escalation
- Five dose cohorts approved with 3 subjects/cohort
- The study period was 360 days
- DSMB assisted in monitoring subjects

# Dosing for AdhAQP1 phase 1 clinical study

AdhAQP1 Clinical Dose Escalation Scheme

Dose Group <sup>*</sup>	Dose in particle units
1	$4.8 \times 10^7$
2	$2.9 \times 10^8$
3	$1.3 \times 10^9$
4	$5.8 \times 10^9$
5	$3.5 \times 10^{10}$

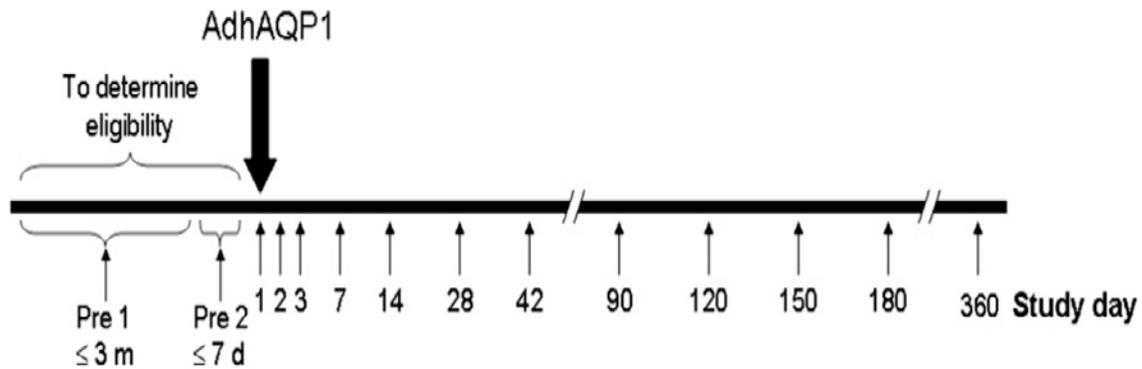
The highest proposed dose has not been associated with any major vector-associated adverse effect and has been well tolerated in previous clinical trials with other tissue targets. It was also well below (<0.1%) the dose of Ad5 vector associated with mortality in a study at the University of Pennsylvania. Dosing was based on a 2.5:1 particles: infectious unit ratio.

Clinical (GMP) vector produced at the Belfer Gene Therapy Core, Cornell University

# Start-up was very slow

- Establishing infrastructure, SOPs, monitoring, database, etc (2007-2008)
- Conservative inclusion and exclusion criteria - of first 50 patients screened (screening begun 7/07) only 4 were eligible, so criteria modified (4/08)
- All modifications took time (i.e., multiple oversight committees)
- Patients high risk for excluding conditions
- Demanding protocol (12 in/out-patient days)

# AdhAQP1 Study Timeline



Modified from Zheng et al, J Gene Med (2010)

# Early responses to adenoviral-mediated transfer of the aquaporin-1 cDNA for radiation-induced salivary hypofunction

Bruce J. Baum<sup>a,1</sup>, Ilias Alevizos<sup>a</sup>, Changyu Zheng<sup>a</sup>, Ana P. Cotrim<sup>a</sup>, Shuying Liu<sup>a</sup>, Linda McCullagh<sup>a</sup>, Corinne M. Goldsmith<sup>a</sup>, Peter D. Burbelo<sup>b</sup>, Deborah E. Citrin<sup>c</sup>, James B. Mitchell<sup>d</sup>, Liesl K. Nottingham<sup>e</sup>, Susan F. Rudy<sup>e</sup>, Carter Van Waes<sup>e</sup>, Millie A. Whatley<sup>f</sup>, Jaime S. Brahim<sup>g</sup>, John A. Chiorini<sup>a</sup>, Stamatina Danielides<sup>a</sup>, R. James Turner<sup>a</sup>, Nicholas J. Patronas<sup>h</sup>, Clara C. Chen<sup>f</sup>, Nikolay P. Nikolov<sup>a</sup>, and Gabor G. Illei<sup>a</sup>

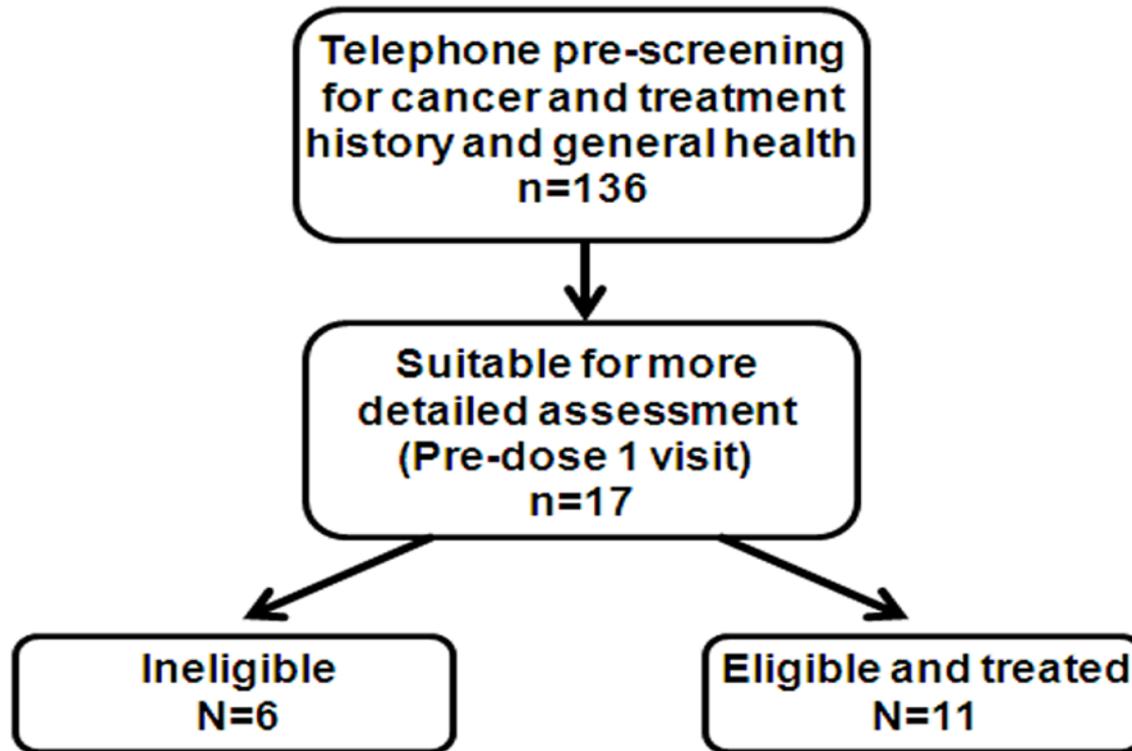
<sup>a</sup>Molecular Physiology and Therapeutics Branch, and <sup>b</sup>Neurobiology and Pain Therapeutics Section, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892; <sup>c</sup>Radiation Oncology Branch and <sup>d</sup>Radiation Biology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; <sup>e</sup>Head and Neck Surgery Branch, National Institute of Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, MD 20892; <sup>f</sup>Nuclear Medicine Section and <sup>h</sup>Section on Neuroradiology, Radiology and Imaging Sciences, Clinical Center, National Institutes of Health, Bethesda, MD 20892; and <sup>g</sup>Department of Oral-Maxillofacial Surgery, University of Maryland, Baltimore, MD 21201

**Proc Natl Acad Sci USA (2012)**

# AdhAQP1 protocol status

- **Eleven** patients received vector (three @  $4.8 \times 10^7$ ; three @  $2.9 \times 10^8$ ; three @  $1.3 \times 10^9$ ; two @  $5.8 \times 10^9$  pu/gland), and enrollment closed (vector reached expiration date in May 2011)
- **All** treated patients tolerated the vector and associated protocol procedures well, i.e., it was safe
- There was objective and subjective evidence of efficacy in one 1<sup>st</sup> cohort patient, two 2<sup>nd</sup> cohort patients and two 3<sup>rd</sup> cohort patients (i.e., **5 of 11 responded positively**)

**Figure 1 Enrollment of Subjects**



Reasons

- difficulty with duct cannulation (4)
- unable to do salivary technetium scans (1)
- new tumor detected (1)

# Characteristics of all treated subjects at baseline

Table 1. Baseline characteristics of all treated subjects

Dose group	Subject #	Age (y)	Sex	Ethnicity	Tumor locale	Tumor stage	Radiation Gy (maximum)	Ad5 NAb	Gland target	Baseline flow (mL/min)	Infusate vol (mL)	vp/ $\mu$ L infused
$4.8 \times 10^7$	40	68	M	C	L tonsil	IVA	66.6	<1:4	Left	tubing*	0.7	$6.86 \times 10^4$
	25	60	M	C	R tonsil	IVA	68.4	<1:1,024	Right	0.162	0.47	$1.02 \times 10^5$
	19	58	M	C	L tonsil	III	50.4	<1:256	Right	0.073	0.76	$6.32 \times 10^4$
$2.9 \times 10^8$	50	58	M	C	BOT	IVA	59.4	<1:8	Left	0.145	0.55	$5.27 \times 10^5$
	73	71	M	C	R tonsil	IVA	69.6	<1:8	Right	0.142	0.7	$4.14 \times 10^5$
	99	53	M	C	R tonsil	IVA	70	<1:128	Left	0.092	0.81	$3.58 \times 10^5$
$1.3 \times 10^9$	105	57	F	C	hypopharynx	IVA	73.8	<1:16,384	Left	0.129	0.95	$1.36 \times 10^6$
	118*	56	M	C	BOT	IVB	71.9	<1:8,192	Left	0.044	0.7	$3.19 \times 10^6$
	103	62	M	C	BOT	IVA	75.4	<1:2,048	Right	0.136	1.8	$7.22 \times 10^5$
$5.8 \times 10^9$	4	53	M	H	L tonsil	IVB	75.6	<1:512	Right	0.085	0.67	$8.66 \times 10^6$
	116	62	M	C	R tonsil	IVA	66.6	<1:8	Left	0.115	0.78	$7.44 \times 10^6$

AdhAQP1 dose is given as vp/gland. For ethnicity, C indicates Caucasian and H indicates Hispanic. For tumor locale, BOT is base of tongue, L is left and R is right. The radiation dose given is the maximum dose to the targeted parotid gland. Ad5 NAb indicates neutralizing antibody titers at baseline (see *SI Methods* for details). Baseline flow from the targeted parotid gland is shown as milliliters per minute. "Tubing" means saliva was in the collection tubing, but was not able to be collected and quantified. For statistical analyses "tubing" was counted as 0.01 mL/min. All subjects experienced late grade 2 toxicity [RTOG classification (2)]. "vol" indicates the volume of the targeted parotid gland measured by contrast radiography (*SI Methods*). The last column to the right indicates the vector particles infused per microliter of infusate, as an in vivo measure of the multiplicity of infection (i.e., vp/tissue mass).

\*Subject #118 received a dose 71.5% higher than intended because of a pharmacy dilution error.

# Adverse Events

**Table 2. Summary of adverse events through day 42**

Dose tier (n)	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)
1 (3)	18*	2	0
2 (3)	19 <sup>†</sup>	3	0
3 (3)	19 <sup>‡</sup>	1	0
4 (2)	3	0	0
Total (%)	59 (90.8)	6 (9.2)	0 (0)

Data shown are the number of adverse events (grades 1, 2, or 3) recorded in each dosing tier. The percentages shown are of the total number (i.e., 65). See footnotes below for specific adverse events; all other adverse events (50/65; 76.9%) were judged as unlikely related or unrelated to treatment.

\*Five were judged possibly related to treatment (subject #s 19, 25, 40).

<sup>†</sup>Three were judged possibly related to treatment (subject #s 73, 99).

<sup>‡</sup>Two were judged possibly, four probably, and one definitely related to treatment (all with subject 105).

**Baum et al, Proc Natl Acad Sci USA (2012)**

# **Transient detection of E1-containing adenovirus in saliva after the delivery of a first-generation adenoviral vector to human parotid gland<sup>†</sup>**

**Changyu Zheng et al**

**Conclusions** The patient most likely had a latent Ad5 infection in the targeted parotid gland that was activated after gene transfer and was without clinical consequence. Published in 2009 by John Wiley & Sons, Ltd.

# Gallium scans: an indicator of inflammation

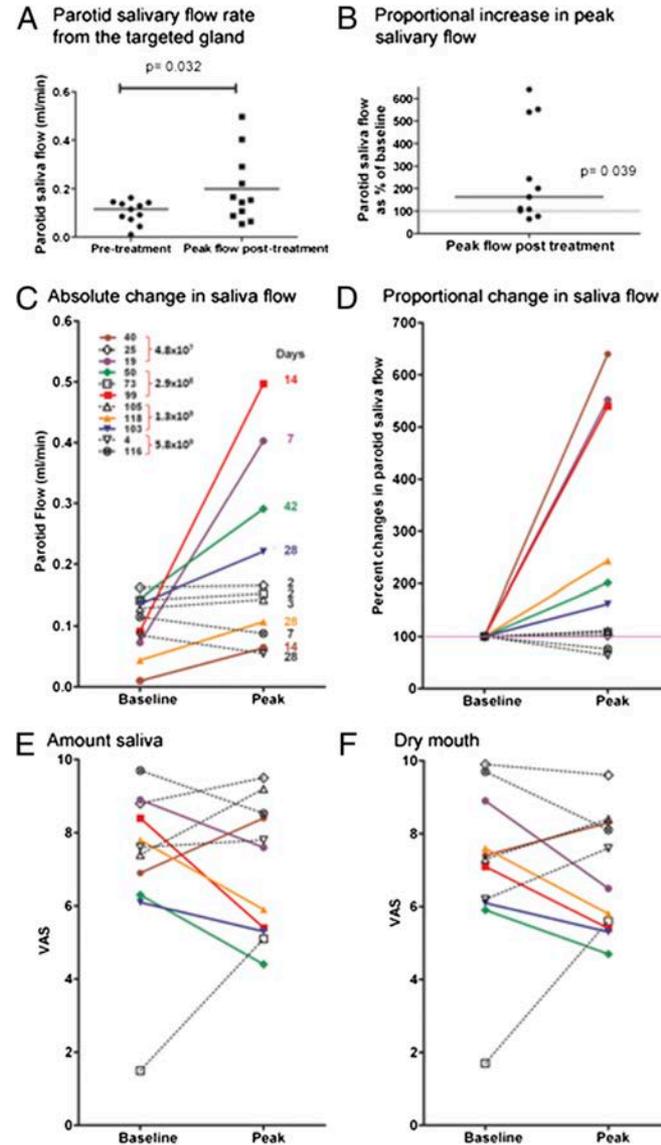
**Table 3. Summary of  $^{67}\text{Ga}$  citrate uptake results**

Dose tier	Subject #	Baseline	+24 h	+96 to 168 h
1	40	1.205	1.168 (0.97)	ND
	25	0.905	0.886 (0.98)	ND
	19	0.941	1.010 (1.07)	ND
2	50	1.250	1.234 (0.99)	ND
	73	0.846	0.884 (1.04)	ND
	99	1.063	1.176 (1.11)	ND
3	105	1.115	1.434 (1.29)	2.720 (2.44)
	118	1.025	0.979 (0.96)	1.151 (1.12)
	103	0.991	1.007 (1.02)	1.124 (1.13)
4	4	0.995	1.483 (1.49)	1.778 (1.79)
	116	1.133	1.381 (1.22)	1.578 (1.39)

Data shown are the results of  $^{67}\text{Ga}$  citrate scans, to assess inflammation in parotid glands, performed at baseline, 24 h or 96–168 h following AdhAQP1 administration to the targeted parotid gland. A region of interest, defining either the targeted or nontargeted contralateral gland, was identified and applied to both glands; the number of counts in each region was quantified, and then the ratio of counts in the targeted/nontargeted gland was calculated (see *SI Methods* for additional details). Next, a ratio of these quotients, at each time-point (+24 h or +96 to 168 h) to that at baseline, was determined. These ratios are in parentheses within the table. ND, not done.

**Baum et al, Proc Natl Acad Sci USA (2012)**

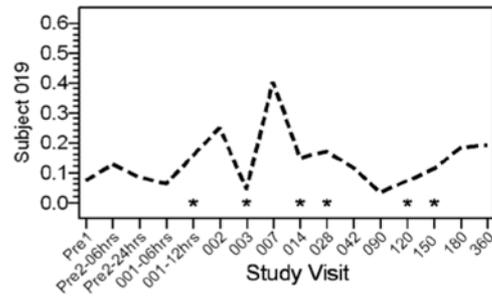
# Effect of AdhAQP1 on Parotid Saliva Flow and Symptoms



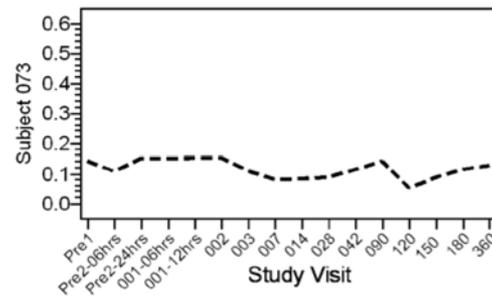
Baum et al, Proc Natl Acad Sci USA (2012)

# Time course of parotid saliva flow rates post-vector delivery

Responder



Non-Responder



### Lymphocyte Proliferation Assay Results (Stimulation Index)

Dose	Subject	Pre	Day 14	Day 28	Day 42	Day 90	Responder
4.8x10 <sup>7</sup>	40	N	N	N	N	N	No
	25	N	N	N	N	N	No
	19	N	3x	2x	3x	1.5x	Yes
2.9x10 <sup>8</sup>	50	N	N	N	N	N	Yes
	73	N	N	----	N	N	No
	99	N	3x	2.5x	2x	5x	Yes
1.3x10 <sup>9</sup>	105	N	8x	4x	4x	6x	No
	118	N	3x	3x	N	2.5x	Yes
	103	N	N	N	N	N	Yes
5.8x10 <sup>9</sup>	4	N	10x	6x	10x	----	No
	116	N	14x	10x	14x	2x	No

**N = within normal limits**

**---- = poor cell sample**

### Ad5 Neutralizing Antibody Levels ( $10^{-1}$ )

Dose	Subject	Pre	Day 2	Day 14	Day 28	Day 120	Day 180	Responder
4.8x10 <sup>7</sup>	40	2	2	2	2	2	2	No
	25	4	8	4	8	16	8	No
	19	64	128	64	64	32	32	Yes
2.9x10 <sup>8</sup>	50	2	2	8	16	2	4	Yes
	73	2	2	2	2	2	2	No
	99	16	8	64	64	16	32	Yes
1.3x10 <sup>9</sup>	105	32768	131072	----	131072	16384	2048	No
	118	8192	16384	32768	8192	1024	2048	Yes
	103	1024	1024	512	2048	2048	1024	Yes
5.8x10 <sup>9</sup>	4	2048	2048	2048	2048	2048	nd	No
	116	2	2	16	16	8	nd	No

# Summary-1

- Gene therapy strategy using AdhAQP1 to treat irradiation-damaged salivary glands was developed and tested in pre-clinical animal models
- Preclinical results showed the strategy to be efficacious and generally safe
- Following approval of a clinical protocol, AdhAQP1 was tested in a Phase 1 study at the NIH Clinical Research Center

# Summary-2

- Gene transfer to human parotid glands is generally safe
- Parotid gland dysfunction can be treated by localized gene transfer
- Positive results in responders did not follow a time course predicted from previous studies in many animal species
- There are useful measurement tools for future studies: parotid flow rate, visual analogue scale for symptom assessment,  $^{67}\text{Ga}$  scans to assess inflammation

# Future studies of hAQP1 gene transfer

- Follow-up study of patients treated with AdhAQP1 (Ilias Alevizos, PI)
- Phase I study of irradiated patients using AAV2hAQP1 (Jay Chiorini, PI; see Gao et al, Gene Ther, 2011)

# COLLEAGUES

## MPTB - "the clinical team"

I. Alevizos A. Cotrim L. McCullagh S. Liu G. Illei  
C. Goldsmith C. Zheng

## Former Gene Transfer Section members

J. Atkinson L. Baccaglini J. Brahim C. Delporte P. Fox  
A. Hoque Y. Marmary A.O'Connell B. O'Connell R. Wellner

## Major collaborators

**Capital Medical University:** Songlin Wang

**NCI:** J. Mitchell, A. Sowers

**NIDCR:** J. Chiorini

**NIEHS:** R. Irwin, M. Vallant