Wang Z, Zourelias L, Wu C, Edwards PC, Trombetta M**, Passineau MJ**. Ultrasound-assisted nonviral gene transfer of AQP1 to the irradiated minipig parotid gland restores fluid secretion. Gene Ther. 2015 Sep;22(9):739-49. doi: 10.1038/gt.2015.36. Epub 2015 Apr 14. PMID: 25871828; PMCID: PMC4560616.

1”groundbreaking Phase 1 human clinical trail using Adenoviral gene transfer or Aquaporin-1 (AQP1) to a single salivary gland from radiation induced xerostiomia –

“unfortunately, the limitations o the Adenoviral vecor system usedin this pioneering trial preclude its advancement to a Phase II trial, and we have thus undertaken to evaluate the therapeutic potential of ultrasound-assisted nonviral gene transfer (UAG) as an alternative means of delivering AQP1 gene therapy to the salivary gland”

Compare head-to-head with canonical Adenoviral vectyor in a swine model.

Irradiated one side – but found sustained hyposalivation that was bilateral.

Treatment with unilateral AQPI gene therapy and UAGT (ultrasound assisted nonviral gene transfer) – bilateral restoration of stimulated salivary flow at 46 h and one week post treatment.

Phase I clinical trial.4,5 This gene therapy strategy is targeted to the ductal cells of the salivary gland, which, in contrast to the saliva-producing acinar cells, are resistant to ionizing radiation and survive radiotherapy largely intact. Using an Adenoviral vector to express

Aquaporin 1 (AdAQP1) in parotid gland ductal cells, Baum et al.5 developed a treatment paradigm that results in the transcellular flux of interstitial fluid across the ductal cell layer and into the intraductal labyrinth of the salivary gland, where the fluid can be expelled to produce palliative oral wetness

***“Despite this remarkable success, a Phase II trial of AdAQP1 is not planned, as the therapeutic effect is transient and this vector is not suitable for the re-administration required to treat this chronic condition.”***

Adenovirus elicits a strong host immune response in humans, and this response is thought to be progressive with repeated exposure.6,7 In aggregate, successful application of AQP1 gene therapy in radiation-induced xerostomia is dependent upon the clinical implementation of a gene therapy technique that evades host immune response and allows for periodic re-administration throughout the lifetime of the individual.

Our efforts toward this goal have focused on ultrasoundassisted gene transfer (UAGT) to the salivary gland, which combines the use of a nonviral DNA vector and lipid/perflutren microbubbles with a low-frequency acoustic field to create a **‘sonoporation’** effect, allowing gene transfer to the cells of the salivary gland without the introduction of viral antigens.

Most notably, this method uses three safe and clinical-grade components: (1) clinical ultrasound; (2) perflutren lipid microbubbles, which have been approved for intravascular administration to humans;10 and (3) plasmid DNA vectors.

10 Definity Package Insert. Available atm http://www.definityimaging.com/pdf/ DEFINITY Prescribing information 515987-0413.pdf. [Prescribing Information for DEFINITY® (definityimaging.com)](https://www.definityimaging.com/pdf/definity-pi.pdf)

Definity perflutren lipd microsphere

DEFINITY Activation, Preparation and Handling Instructions Follow directions for activation of DEFINITY carefully and adhere to strict aseptic procedures during preparation. 1. Allow the vial to warm to room temperature before starting the activation procedure. 2. Activate DEFINITY by shaking the vial for 45 seconds using a VIALMIX device or VIALMIX RFID device. Note: illustrations of this procedure are contained in the VIALMIX or VIALMIX RFID User’s Guide. Do not use this drug unless it has completed a full 45 second activation cycle in the VIALMIX or VIALMIX RFID. DEFINITY will not be properly activated unless the full 45 second activation cycle is completed. Error messages will display if the vial is not properly activated. Do not reactivate the vial if VIALMIX or VIALMIX RFID did not properly activate the vial. Never reactivate a successfully activated DEFINITY vial (see step 3). A VIALMIX or VIALMIX RFID that is not functioning properly must never be used. Only use a vial activated from a properly functioning VIALMIX or VIALMIX RFID. Refer to the VIALMIX or VIALMIX RFID User’s Guide to ensure that a properly functioning VIALMIX or VIALMIX RFID is used.

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DEFINITY® may be used for up to 12 hours after activation with VIALMIX® or VIALMIX® *RFID*. If an activated vial is not used within 5 minutes, resuspend by hand agitation for 10 seconds prior to use.1

[Proper Activation, Flexible Dosing, and Administration of DEFINITY® (definityimaging.com)](https://www.definityimaging.com/using-definity)

CONTRAINDICATIONS

Do not administer DEFINITY® and DEFINITY® RT to patients with known or suspected hypersensitivity to perflutren lipid microsphere or its components, such as polyethylene glycol (PEG).

1. Mulvagh SL, Rakowski H, Vannan MA, et al. American society of echocardiography consensus statement on the clinical applications of ultrasonic contrast agents in echocardiography. *J Am Soc Echocardiogr.* 2008;21(11):1179-1281.

**For UAGT, Definity microbubbles (Lantheus Medical Imaging, North Billerica, MA, USA) were activated per manufacturer’s instructions immediately before the procedure and mixed with 1 mg ml − 1 plasmid vector (either pCMV-pAQP1 or pCMV-MetLuc) before being added to the infusate (total dose 3 mg). Immediately after infusion, a layer of clinical ultrasound gel was applied to the skin overlying the parotid gland, and SoniGene ultrasound beam emitter (VisualSonics, Toronto, Canada) was applied to the skin and moved over the outline of the parotid gland using a gliding motion. A total ultrasound exposure of 4 treatments comprising 30 sec each, 2 W cm− 2 at a 50% duty cycle with 10 sec between treatments was delivered. Following gene transfer, the animal was allowed to awaken and returned to its housing.**

Definity Package Insert. Available atm http://www.definityimaging.com/pdf/ DEFINITY Prescribing information 515987-0413.pdf.

This method has been shown to successfully express transgenes within the salivary gland of rodents,8,9

8 Passineau MJ, Zourelias L, Machen L, Edwards PC, Benza RL. Ultrasound-assisted non-viral gene transfer to the salivary glands. Gene Therapy 2010; 17: 1318–1324. 9 Geguchadze R, Wang Z, Zourelias L, Perez-Riveros P, Edwards P, Machen L et al. Proteomic profiling of salivary gland after nonviral gene transfer mediated by conventional plasmids and minicircles. Mol Ther Methods Clin Dev 2014; 1: 14007.