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### Authors

Chen, Xiaolan  
Lee, Sujin  
Zhang, Tianyi  
[et al.](#)

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# Nanomolar Potency Aminophenyltriazine CFTR Activator Reverses Corneal Epithelial Injury in a Mouse Model of Dry Eye

Xiaolan Chen,<sup>1,2</sup> Sujin Lee,<sup>1</sup> Tianyi Zhang,<sup>1</sup> Tianying Duan,<sup>1</sup> Neel D. Pasricha,<sup>3</sup> Julie M. Schallhorn,<sup>3</sup> Marc H. Levin,<sup>4</sup> Vuk Koprivica,<sup>5</sup> and Alan S. Verkman<sup>1</sup>

## Abstract

**Purpose:** Dry eye disorders are a major health care burden. We previously reported the identification of *N*-methyl-*N*-phenyl-6-(2,2,3,3-tetrafluoropropoxy)-1,3,5-triazine-2,4-diamine [cystic fibrosis transmembrane conductance regulator (CFTR)<sub>act</sub>-K267], which activated human wild-type CFTR chloride conductance with EC<sub>50</sub> ~ 30 nM. Here, we report *in vivo* evidence for CFTR<sub>act</sub>-K267 efficacy in an experimental mouse model of dry eye using a human compatible ophthalmic vehicle.

**Methods:** CFTR activation in mice *in vivo* was demonstrated by ocular surface potential difference (OSPD) measurements. Ocular surface pharmacodynamics was measured in tear fluid samples obtained at different times after topical administration of CFTR<sub>act</sub>-K267. Dry eye was produced by lacrimal duct cauterization (LDC) and corneal epithelial injury and was assessed by Lissamine green (LG) staining.

**Results:** OSPD measurements demonstrated a hyperpolarization of  $-8.6 \pm 3$  mV (standard error of the mean, 5 mice) in response to CFTR<sub>act</sub>-K267 exposure in low chloride solution that was reversed by a CFTR inhibitor. Following single-dose topical administration of 2 nmol CFTR<sub>act</sub>-K267, tear fluid CFTR<sub>act</sub>-K267 concentration was >500 nM for more than 6 h. Following LDC, corneal surface epithelial injury, as assessed by LG staining, was substantially reversed in 10 of 12 eyes receiving 2 nmol CFTR<sub>act</sub>-K267 3 times daily starting on day 2, when marked epithelial injury had already occurred. Improvement was seen in 3 of 12 vehicle-treated eyes.

**Conclusion:** These studies provide *in vivo* evidence in mice for the efficacy of a topical, human use compatible CFTR<sub>act</sub>-K267 formulation in stimulating chloride secretion and reversing corneal epithelial injury in dry eye.

**Keywords:** keratoconjunctivitis sicca, CFTR, chloride channel, lacrimal duct cauterization

## Introduction

**D**RY EYE DISORDERS are a major health care burden, with an estimated prevalence of 16 million people in the United States and affecting more than 10% of people older than 50 years.<sup>1,2</sup> While heterogeneous by etiology, dry eye disease presents with common clinical signs and symptoms, which include corneal epithelial injury as assessed by fluorescein and Lissamine green (LG) staining, decreased ocular fluid and tear breakup time, and patient-reported symptoms of ocular discomfort or functional impairment in a low humidity environment. Treatment options available in the United States at present include artificial tears, punctal

plugs, and the topical anti-inflammatory drugs cyclosporine and lifitegrast.<sup>3-6</sup> Despite available options, dry eye disease is increasing in incidence and remains an unmet medical need.

We previously proposed prosecretory therapy for dry eye by targeting cystic fibrosis transmembrane conductance regulator (CFTR), a secretory chloride channel expressed in corneal and conjunctival epithelial cells at the ocular surface.<sup>7-9</sup> As in the intestine in secretory diarrhea, activation of CFTR in cornea and conjunctiva can produce a secretory chloride current that results in net fluid secretion onto the ocular surface. Restoration of the normal aqueous layer in dry eye is predicted to reduce tear fluid hyperosmolality, a

Departments of <sup>1</sup>Medicine and Physiology and <sup>3</sup>Ophthalmology, University of California, San Francisco, California.

<sup>2</sup>Jiangsu Agri-Animal Husbandry Vocational College, Taizhou, China.

<sup>4</sup>Department of Ophthalmology, Palo Alto Medical Foundation, Palo Alto, California.

<sup>5</sup>Vanda Pharmaceuticals, Inc., Washington, District of Columbia.

major driver of ocular surface inflammation and clinical symptoms, and could thus be an effective strategy to treat a broad spectrum of dry eye diseases.

We previously identified, by high-throughput screening, the aminophenyl-1,3,5-triazine CFTR<sub>act</sub>-K089, which activated human wild-type CFTR with EC<sub>50</sub> ~ 250 nM and produced a secretory response at ocular surface epithelia in mice.<sup>10</sup> Utilizing medicinal chemistry, we discovered analog *N*-methyl-*N*-phenyl-6-(2,2,3,3-tetrafluoropropoxy)-1,3,5-triazine-2,4-diamine (CFTR<sub>act</sub>-K267), which activated CFTR chloride conductance with EC<sub>50</sub> ~ 30 nM, underwent rapid hepatic metabolism (minimizing systemic exposure), and produced a sustained increase in tear volume in mice when administered topically in a dimethyl sulfoxide (DMSO)-containing ophthalmic formulation.<sup>11</sup> CFTR<sub>act</sub>-K267 in the DMSO-containing formulation also produced a sustained secretory response in rabbits without tachyphylaxis upon 28-day repeated dosing and was detected mainly at the ocular surface without detectable blood levels.<sup>12</sup>

The purpose of this study was to test the efficacy of CFTR<sub>act</sub>-K267, delivered in an ophthalmic vehicle suitable for use in humans, in an experimental animal model of dry eye produced by lacrimal duct cauterization (LDC) in mice. The prosecretory action of CFTR<sub>act</sub>-K267 *in vivo* was studied by measurement of ocular surface potential differences (OSPDs), a method originally developed by our laboratory,<sup>9</sup> and its ocular surface pharmacokinetics was measured by liquid chromatography/mass spectroscopy (LC/MS) analysis of collected tear fluid.

## Methods

### Mice

Female Balb/c mice (aged 7–8 weeks) were bred at the University of California, San Francisco (UCSF) Animal Facility. Animal protocols were approved by the UCSF Institutional Animal Care and Use Committee and were in compliance with the Association for Research in Vision and Ophthalmology statement for the use of animals in ophthalmic and vision research.

### Chemicals

CFTR<sub>act</sub>-K267 was synthesized by sequential substitution reactions of cyanuric chloride with *N*-methylamine, 2,2,3,3-tetrafluoropropanol, and aniline under basic conditions, as described.<sup>11</sup> CFTR<sub>act</sub>-K267 was purified by flash column chromatography (n-hexane:ethylacetate 1:2), giving purity >98% as determined by LC/MS. Other chemicals were purchased from Sigma–Aldrich (St. Louis, MO).

### Ophthalmic formulation

CFTR<sub>act</sub>-K267 (10 μmol, 3.4 mg) was dissolved in 10 mL of a human compatible ophthalmic vehicle that completely dissolved the compound. The vehicle contained 50 mg/mL polyoxyl 35 castor oil, 10 mg/mL polyethylene glycol-400, 10 mg/mL propylene glycol, 2 mg/mL NaCl, and 2 mg/mL sodium carboxymethyl cellulose, which was brought up to a volume of 1 mL with pH 7.4 phosphate buffer (prepared from 0.275 mg NaH<sub>2</sub>PO<sub>4</sub> monohydrate and 1.137 Na<sub>2</sub>PO<sub>4</sub> anhydrous in 1 mL water, titrated to pH 7.4 with 0.1 M phosphoric acid).

### OSPD measurements

Open-circuit transepithelial potential difference (PD) in millivolts (mV) at the ocular surface was measured in anesthetized mice as described.<sup>13</sup> Mice were anesthetized with isoflurane, and core temperature was maintained at 37°C with a heating pad. The eyes were oriented with the cornea and conjunctiva facing upward. Solutions were isosmolar (320 ± 10 mOsM) with a pH of 7.4, consisting of (in mM): solution 1: 142 NaCl, 3 KCl, 0.8 NaH<sub>2</sub>PO<sub>4</sub>, 1 CaCl<sub>2</sub>, 0.5 MgCl<sub>2</sub>, 1.5 KH<sub>2</sub>PO<sub>4</sub>; solution 2: solution 1 + amiloride (100 μM); solution 3: (low Cl<sup>-</sup>) solution 2 with NaCl replaced by Na gluconate and KCl by K gluconate; solution 4: solution 3 + CFTR<sub>act</sub>-K267 (2.5 or 10 μM); solution 5: solution 4 + forskolin (20 μM); solution 6: solution 5 + CFTR<sub>inh</sub>-172 (10 μM). The ocular surface was perfused at 6 mL/min through plastic tubing with a multireservoir gravity pinch-valve system and variable-flow peristaltic pump. A probe catheter was immobilized 1 mm above the cornea with a micropositioner, and a suction cannula was positioned 3 mm from the orbit. The measuring electrode contacting the perfusion catheter was connected to a high-impedance voltmeter (IsoMilivolt Meter; World Precision Instruments), with reference consisting of a 21-gauge needle filled with isosmolar saline inserted subcutaneously. Measuring and reference electrodes consisted of Ag/AgCl with 3 M KCl agar bridges.

### Ocular pharmacokinetics

Mice were topically given 2 μL of 1 mM CFTR<sub>act</sub>-K267 in ophthalmic vehicle by micropipette. At specified times, 3 eye washes [3 μL phosphate-buffered saline (PBS) each] were recovered from the lateral and medial canthi using 5 μL microcapillary tubes (Drummond Scientific Co., Broomhall, PA) after manual eyelid blinking. 6 μL of pooled washes were mixed with 34 μL of eluent solution (1:1 mixture of acetonitrile/water containing 0.1% formic acid) and analyzed by LC/MS. LC was performed on a Xterra MS C18 column (2.1 × 100 mm, 3.5 μm) with 0.2 mL/min water/acetonitrile containing 0.1% formic acid, 24-min linear gradient, and 5% to 95% acetonitrile connected to a 2695 HPLC (Waters, Milford, MA) solvent delivery system. Low-resolution MS was performed on a Micromass ZQ mass spectrometer (Waters) with positive electrospray ionization.

### LDC model

A LDC model was used as described,<sup>10</sup> modified from Stevenson et al.<sup>13</sup> On day 0, the extraorbital lacrimal glands were exposed bilaterally by 3-mm linear skin incisions. The linear duct was reliably visualized along its course to the lateral canthus by gently lifting the main lacrimal gland from the face with blunt forceps. The lacrimal duct on each side was ablated with a high-temperature handheld cautery pen, avoiding facial vessels and nerves. Incisions were closed with a single, interrupted 6-0 silk suture. Accessory lacrimal tissue remained intact. On day 2 (and in some studies on day 0 just before LDC), to assess corneal epithelial integrity, 5 μL of 1% LG dye was applied to the ocular surface of isoflurane-anesthetized mice and washed after 10 s with PBS. Two photographs of each eye were taken with a digital camera (Canon, Revel T5i, Japan) adapted to a zoom stereo microscope (Nikon, SMZ1500, Japan). After

LG staining on day 2, mouse eyes were randomized to receive CFTR<sub>act</sub>-K267 treatment in one eye and vehicle in the other eye. Treatment was 2 nmol CFTR<sub>act</sub>-K267 in 2  $\mu$ L ophthalmic vehicle given 3 times per day (at 9 AM, 2 PM, and 7 PM) for 5 days starting on day 2. LG staining was also performed on days 4 and 6 at 2 h after the 7 PM eye drop.

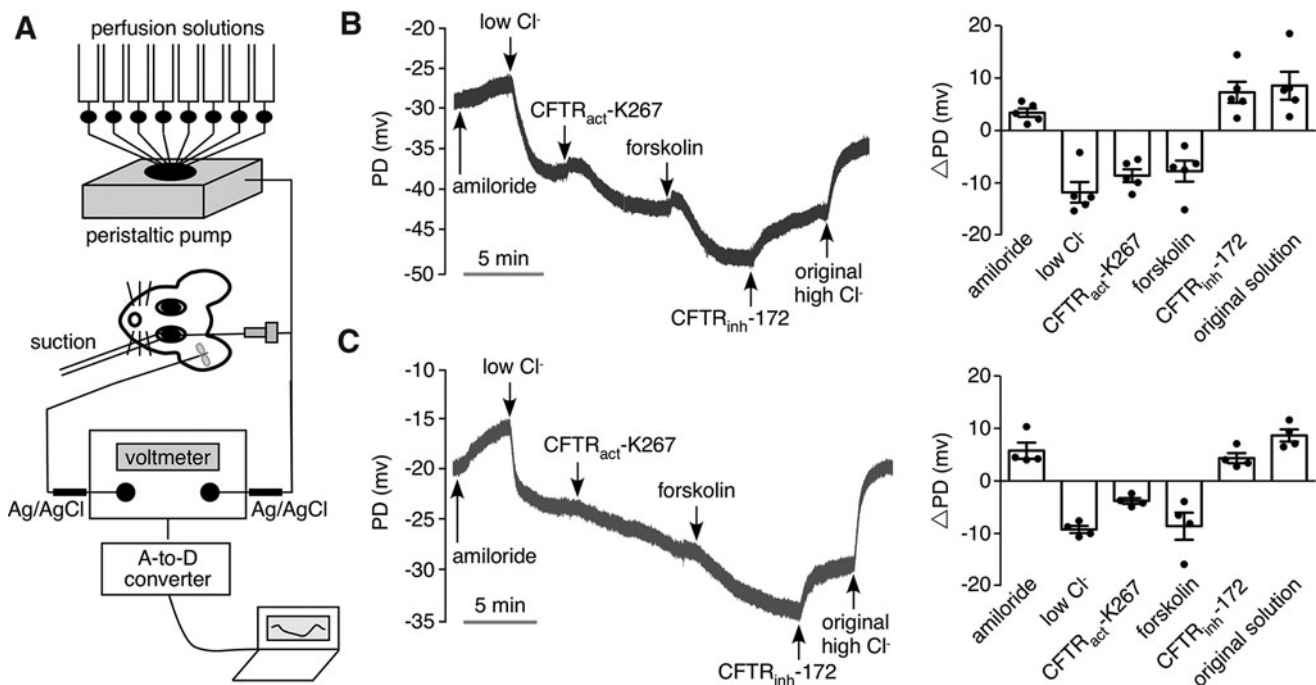
### Data analysis and statistics

Photographs from LG staining were scored by 2 investigators who were unaware of treatment status, with each grader using a different scoring method. In the first scoring method, each corneal quadrant was scored on a 3-point scale with staining in each quadrant classified as: grade 0, no staining; grade 1, sporadic staining (involving <25% of the total surface); grade 2, diffuse punctate staining (25%–75%); grade 3, coalesced punctate staining ( $\geq$ 75%). The total “Lissamine green score” was determined as the sum of scores from all four quadrants, ranging from 0 to 12. In the second scoring method giving a “Comparative Lissamine green grade,” photos from days 2, 4, and 6 were scored comparatively, comparing R versus L eyes in each mouse (0, no difference; +1, R eye with mildly more staining than L eye; +2, R eye with much more staining than left eye; –1 and –2, R eye with mildly and much less staining than L eye). Data are presented as mean  $\pm$  standard error of the mean (SEM). Statistical analyses were performed using GraphPad prism 8 software with statistical comparisons made using the paired 2-tailed *t*-test.

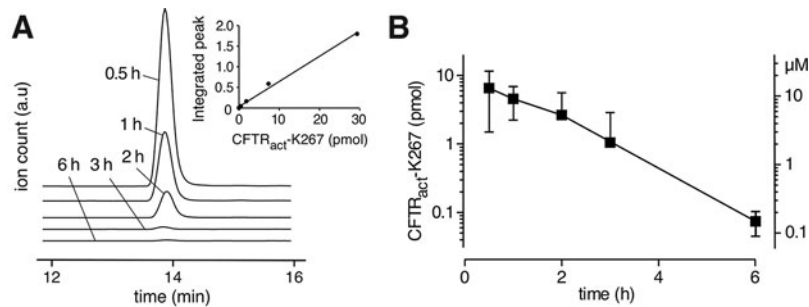
## Results

### OSPD measurement

CFTR<sub>act</sub>-K267 activation of CFTR chloride conductance at the ocular surface was studied in live mice using an ocular surface PD measurement method in which the PD (in mV) generated by the ocular surface epithelium *in situ* was measured using a high-impedance voltmeter with sensing electrodes in electrical contact with the ocular surface and body (Fig. 1A). Initial ocular surface PD measured using a physiological chloride-containing solution in the perfusate (solution 1, see the Methods section) was  $-30 \pm 8$  mV (SEM,  $n=5$  eyes), which depolarized by  $3.4 \pm 1.8$  mV following addition of amiloride (solution 2) to inhibit the epithelial sodium channel (ENaC). A representative curve is shown at the left and data from separate experiments along with averaged values for PD changes ( $\Delta$ PD) are shown on the right. Then, perfusion with a low chloride solution containing amiloride (solution 3) produced a hyperpolarization in part due to a diffusion potential. Perfusion with CFTR<sub>act</sub>-K267 at 10  $\mu$ M (solution 4), followed by the CFTR agonist forskolin (solution 5) produced hyperpolarizations of  $8.6 \pm 2.7$  mV and  $7.8 \pm 4.5$  mV, respectively. Perfusion with solution containing the CFTR inhibitor CFTR<sub>inh</sub>-172 (solution 6) resulted in a depolarization of  $7.3 \pm 4.5$  mV. Figure 1C shows a similar study but with 2.5  $\mu$ M CFTR<sub>act</sub>-K267, a concentration chosen from pilot studies as producing a robust response. A slow  $3.0 \pm 1.0$  mV hyperpolarization was seen with 2.5  $\mu$ M CFTR<sub>act</sub>-K267 in the low chloride solution. The results



**FIG. 1.** CFTR<sub>act</sub>-K267 activation of CFTR chloride conductance at the ocular surface in mice studied by ocular surface PD measurements. (A) Schematic of the method showing perfusion of the ocular surface with different solutions and measurement of PD using a high-impedance voltmeter, with sensing electrode in electrical contact with the perfusion solution and grounding needle electrode placed under the skin. (B, C) (Left) Representative continuous recordings of ocular surface PD as a function of time in response to perfusion with solutions containing indicated compounds (see the Methods section for solution compositions). Concentrations of CFTR<sub>act</sub>-K267 were 10  $\mu$ M (B) and 2.5  $\mu$ M (C). (Right) Summary of changes in ocular surface PD ( $\Delta$ PD) for successive solution changes. Each point is from a study performed on a different eye (mean  $\pm$  SEM). CFTR, cystic fibrosis transmembrane conductance regulator; PD, potential difference; SEM, standard error of the mean.



**FIG. 2.** Ocular pharmacokinetics of CFTR<sub>act</sub>-K267. **(A)** LC/MS assay of CFTR<sub>act</sub>-K267 amount in tear fluid at 0.5, 1, 2, 3, and 6 h after single-dose (2 nmol) administration as 2  $\mu$ L eye drop. Representative chromatograms ( $m/z=332$ ) shown, with standard curve shown as *inset*. **(B)** Averaged recovered CFTR<sub>act</sub>-K267 (*left ordinate*) and deduced CFTR<sub>act</sub>-K267 concentration (*right ordinate*) computed assuming a 0.5  $\mu$ L tear fluid volume per eye (mean  $\pm$  SEM, 4 eyes per time point). LC/MS, liquid chromatography/mass spectroscopy.

provide *in vivo* evidence for CFTR<sub>act</sub>-K267 activation of CFTR chloride conductance at the ocular surface.

#### Tear fluid pharmacokinetics

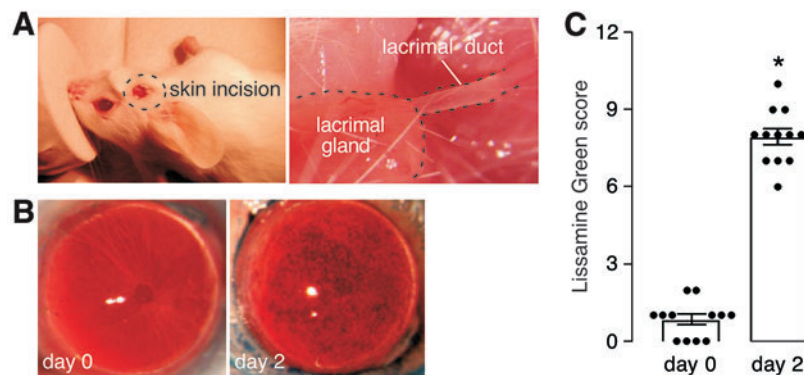
The amount of CFTR<sub>act</sub>-K267 in tear fluid at different times after single-dose topical administration of 2 nmol CFTR<sub>act</sub>-K267 was determined by LC/MS analysis of combined fluid from 3 eye washes, using different mice for each time point. Figure 2A (*inset*) shows an approximately linear standard curve for LC/MS integrated peak ion current as a function of CFTR<sub>act</sub>-K267 amount from which CFTR<sub>act</sub>-K267 in the combined eye washes was determined. Figure 2A also shows original LC/MS curves for recovered CFTR<sub>act</sub>-K267 per eye at different times after eye drop administration. Averaged results from 4 eyes per time point showed an approximately exponential reduction in CFTR<sub>act</sub>-K267 amount with time, with computed  $t_{1/2}$  of 62 min (Fig. 2B). The deduced tear film concentration of CFTR<sub>act</sub>-K267, computed assuming a tear fluid volume of 0.5  $\mu$ L per eye,<sup>14</sup> is shown on the right-hand ordinate. The estimated CFTR<sub>act</sub>-K267 concentration was well above its EC<sub>50</sub> of  $\sim$ 30 nM, as measured in cell studies,<sup>11</sup> for at least 6 h after single-dose administration. The amounts and deduced concentrations of CFTR<sub>act</sub>-K267 may underestimate the

actual values by 15%–20% based on the recovery efficiency of 80%–85% estimated previously using a fluorescent fluid-phase marker.<sup>10</sup>

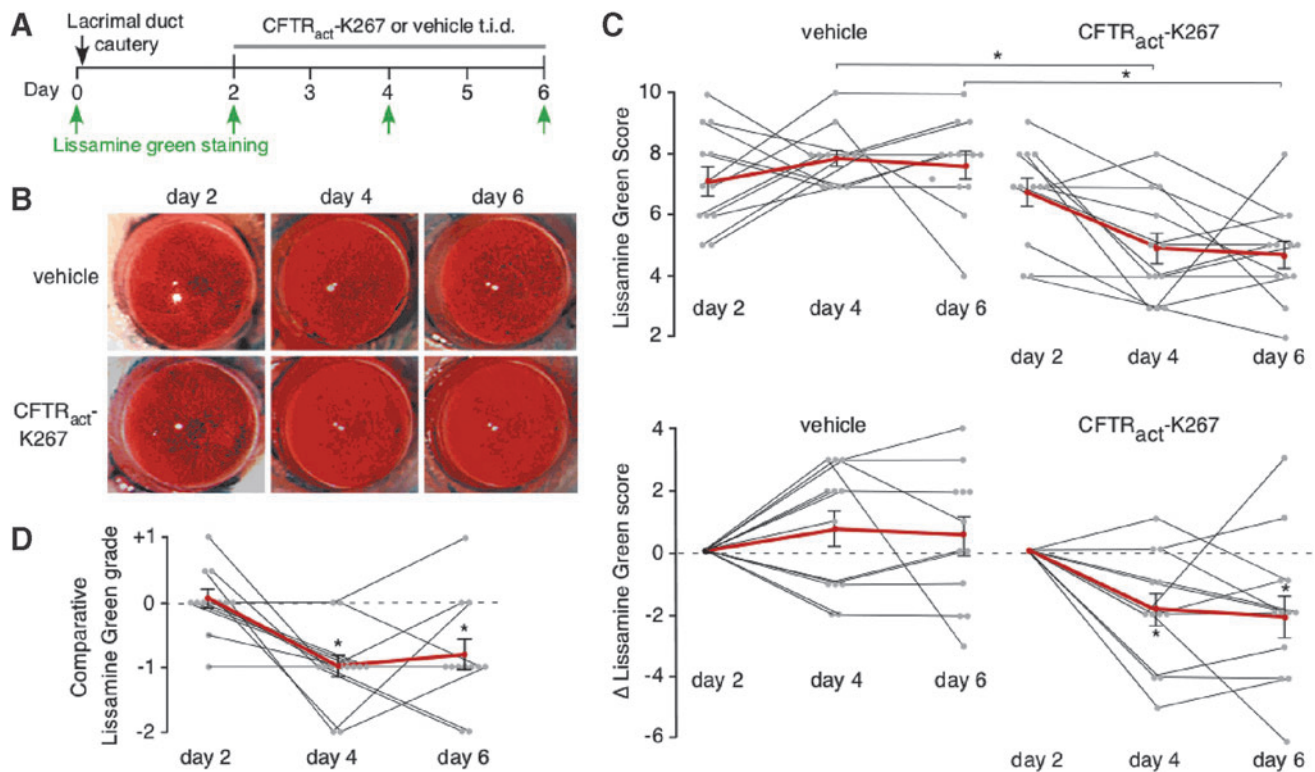
#### LDC model of dry eye

A robust LDC model was implemented in mice in which cauterization of the main lacrimal duct, located between the inferior ear lobe and the lateral canthus, resulted in consistent corneal epithelial injury as determined by LG staining (Fig. 3A). Representative photographs of mouse corneas show little LG staining before LDC, with marked staining of the same ocular surfaces at 2 days after LDC (Fig. 3B). No increase in LG staining was seen in sham-operated eyes in which the cauterization step was omitted (data not shown). Figure 3C summarizes corneal epithelial injury scores before and 2 days after LDC in 12 eyes studied. All operated eyes show moderate epithelial disruption within a narrow range.

To study the efficacy of CFTR<sub>act</sub>-K267, delivered using an optimized ophthalmic vehicle, in reversing corneal epithelial injury, the formulation studied in Fig. 2 was administered to mice 3 times daily beginning after the LG staining on day 2, with LG staining repeated on days 4 and 6 (Fig. 4A). For each mouse, 1 eye was administered 2  $\mu$ L of CFTR<sub>act</sub>-K267-containing formulation (2 nmol) and the



**FIG. 3.** LDC model of dry eye disease in mice. **(A)** LDC was produced by high-temperature cauterization of the main lacrimal duct bilaterally, identified following a short linear skin excision to expose the main lacrimal gland and its duct. **(B)** Representative photos of LG staining of cornea just before and 2 days after bilateral LDC. **(C)** Scoring of corneal epithelial injury deduced from photographs as in **(B)** using a 12-point standard scale (see the Methods section) (12 eyes, mean  $\pm$  SEM,  $*P < 0.001$ ). LDC, lacrimal duct cauterization; LG, Lissamine green. Color images are available online.



**FIG. 4.** Topical CFTR<sub>act</sub>-K267 reverses corneal epithelial injury in mice following LDC. **(A)** Experimental protocol. **(B)** Representative photos of LG staining of cornea at days 2, 4, and 6 after LDC in vehicle and CFTR<sub>act</sub>-K267-treated eyes. **(C)** Photos scored using a 12-point standard scale (see the Methods section). *(Top)* Absolute LG scores on day 2 (2 days after LDC, before treatment) days 4 and 6 in vehicle and CFTR<sub>act</sub>-K267-treated eyes (mean  $\pm$  SEM, 12 eyes,  $*P < 0.01$ , by *t*-test). *(Bottom)* Data shown for each individual eye (*black lines*) as the change in LG scores from those on day 2. Averaged data are shown as *red lines* (mean  $\pm$  SEM, 12 eyes,  $*P < 0.05$  compared with day 2, by *t*-test). **(D)** Photos scored using a comparative grading method (see the Methods section). Data shown for individual mice as Comparative LG grade for CFTR<sub>act</sub>-K267- versus vehicle-treated eyes (mean  $\pm$  SEM, 12 eyes,  $*P < 0.01$  compared with day 2, by *t*-test). Color images are available online.

other eye was administered control vehicle, with the treated eye (left vs. right) randomized. A total of 12 mice (6 per group) were studied. Representative LG photographs of CFTR<sub>act</sub>-K267-treated and contralateral vehicle-treated eyes are shown in Fig. 4B.

LG photographs of corneas were independently scored by 2 investigators (M.H.L. and A.S.V.) who were unaware of eye treatment status and using different scoring systems. Utilizing a standard 12-point scoring system that summed staining in each quadrant of each cornea,<sup>15</sup> there was a significant reduction in staining score in the CFTR<sub>act</sub>-K267-treated eyes at days 4 and 6, shown as absolute staining scores (Fig. 4C, top) or as the difference ( $\Delta$ ) in staining score (Fig. 4C, bottom). No significant change in staining score was seen in the vehicle-treated eyes. Improvement in score was seen in 10 of 12 of the CFTR<sub>act</sub>-K267 treated eyes. Using an independent, comparative grading system in which LG staining in left and right eyes was compared in each mouse, there was a significant improvement in the “Comparative Lissamine green score” on days 4 and 6, with improvement seen in 10 of 12 CFTR<sub>act</sub>-K267-treated eyes on day 4 and in 9 of 12 on day 6 (Fig. 4D).

## Discussion

The data herein provide evidence for the efficacy of aminophenyltriazine CFTR<sub>act</sub>-K267 in activating CFTR

chloride conductance at the ocular surface in mice and in reversing corneal epithelial injury in a surgical mouse model of aqueous-deficient dry eye. For these studies, a DMSO-free ophthalmic formulation was established for stable dissolution and topical ocular delivery of CFTR<sub>act</sub>-K267, which by ocular pharmacokinetics measurements produced predicted therapeutic levels of CFTR<sub>act</sub>-K267 in the tear film for at least 6 h following single-dose administration. CFTR<sub>act</sub>-K267 efficacy in producing a CFTR-mediated chloride secretory response at the ocular surface in mice was demonstrated by open-circuit PD measurements in which the addition of CFTR<sub>act</sub>-K267 in a low chloride-containing solution resulted in a hyperpolarization of magnitude comparable to that produced by the cAMP agonist forskolin. A mouse model of dry eye involving LDC showed consistent and robust corneal epithelial injury 2 days after cauterization, which was largely reversed by topical CFTR<sub>act</sub>-K267.

The corneal epithelium transports chloride from the basal side to the tear side to maintain corneal hydration and clarity.<sup>16</sup> Expression of several chloride transporters has been reported in corneal epithelial cells, including CLCA2, a putative calcium-activated chloride channel seen in human and chick corneal epithelial cells,<sup>17</sup> and CFTR in mouse, rabbit, and human cornea.<sup>10,12,18</sup> In the eye, CFTR is also expressed in corneal endothelium and retinal pigment epithelium, where it has been proposed to facilitate corneal deturgescence<sup>19</sup> and regulate subretinal extracellular volume

and ionic content.<sup>20,21</sup> CFTR has also been localized to apical superficial cell membranes of the stratified conjunctival epithelium<sup>22,23</sup> and in lacrimal gland epithelium,<sup>7</sup> suggesting a role in tear film homeostasis and as a target for therapy of dry eye.

A prosecretory therapeutic is anticipated to be beneficial in dry eye disease from a variety of etiologies such as aqueous deficiency, as seen in Sjögren's syndrome, but also in evaporative dry eye states.<sup>24,25</sup> Increasing tear fluid volume on the ocular surface would reduce tear hyperosmolality and the downstream inflammatory response. Currently, 5% lifitegrast ophthalmic solution is Food and Drug Administration approved to treat dry eye in the United States. 0.05% cyclosporine ophthalmic emulsion, which is also routinely used to treat dry eye, is formally approved to normalize tear secretion, although by an uncertain mechanism. These are both anti-inflammatory drugs that limit the ocular surface inflammatory response common in dry eye patients. In Japan, 3% diquafosol ophthalmic solution, which stimulates a prosecretory response through the P2Y2 purinergic receptor, and 2% rebamipide ophthalmic suspension, which has a stabilizing effect on the ocular surface mucin layer, have been approved.<sup>26</sup> Other therapeutic approaches for addressing dry eye are being investigated.<sup>27</sup>

Our original studies were performed with a vehicle containing DMSO,<sup>11</sup> which can solubilize a wide variety of otherwise poorly soluble polar and nonpolar molecules. With the goal of translating a prosecretory CFTR-activating compound to human clinical trials, a non-DMSO-containing formulation, incorporating ocular dosing-approved vehicles, was evaluated here. CFTR<sub>act</sub>-K267 was stably dissolved in the formulation, which when delivered topically to mice showed favorable pharmacokinetics and efficacy in reversing dry eye. The human compatible CFTR<sub>act</sub>-K267 formulation reversed the effects of lacrimal gland cautery in the majority of treated eyes following 2 days of treatment, and a single dose gave ocular fluid concentrations that are predicted to be sufficient for CFTR activation over at least 6 h. The data suggest that, if it passes safety testing, CFTR<sub>act</sub>-K267 could be a potential treatment for dry eye associated with decreased ocular tear film. These results further support that potential utility of stimulating CFTR secretory activity on the ocular surface for the treatment of dry eye disease.

In summary, CFTR<sub>act</sub>-K267 in a human use compatible ophthalmic formulation increased chloride secretion at the ocular surface and reversed corneal epithelial injury in an experimental mouse model of dry eye. Ocular fluid pharmacokinetics studies showed predicted therapeutic concentrations of CFTR<sub>act</sub>-K267 for at least 6 h following single-dose topical administration, supporting further development of CFTR<sub>act</sub>-K267 as a candidate dry eye therapeutic.

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## Authors' Contributions

M.H.L., V.K., and A.S.V. designed experiments. T.Z. and T.D. performed potential difference measurements. X.C. and S.L. performed pharmacokinetic studies. X.C. performed

lacrimal duct ligation studies. M.H.L. and A.S.V. analyzed lacrimal gland ligation studies. All authors contributed to writing of the article.

## Author Disclosure Statement

S.L., M.H.L., and A.S.V. are named inventors on patent applications on CFTR activators for dry eye owned by the University of California, San Francisco. M.H.L. and A.S.V. are consultants for Vanda Pharmaceuticals, Inc., and V.K. is an employee of Vanda Pharmaceuticals, Inc. The CFTR<sub>act</sub>-K267 patents are licensed to Vanda Pharmaceuticals, Inc. The other authors have nothing to disclose.

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Address correspondence to:  
Dr. Alan S. Verkman  
1246 Health Sciences East Tower  
University of California, San Francisco  
513 Parnassus Avenue  
San Francisco, CA 94143-0521

E-mail: alan.verkman@ucsf.edu

Dr. Vuk Koprivica  
Vanda Pharmaceuticals, Inc.  
2200 Pennsylvania Avenue Northwest, Suite 300E  
Washington, DC 20037

E-mail: vuk.koprivica@vandapharma.com