Effects of Nectin-4 targeted Bicycle® Toxin Conjugate, zelenectide pevedotin (BT8009), and enfortumab vedotin on a human corneal tissue model

Abstract #

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Julia Kristensson, Carly Campbell*, Steve Ludbrook, Sandra Hirschberg and Mike Rigby
BicycleTx, Portway Building, Granta Park, Cambridge CB21 6GS, United Kingdom
*BicycleTx, 35 Cambridge park Drive, Cambridge, MA, 02140, United States

ABSTRACT

- Bicycle Toxin Conjugates® are a new therapeutic class in development that offers the manufacturing and pharmacokinetic properties of a small molecule with the high binding specificity of a biologic, making them ideally suited for targeted delivery of cytotoxins to solid tumours (1, 2).
- Zelenectide pevedotin (zele; previously BT8009) is a BTC® molecule comprised of a highly selective Nectin-4-targeted Bicycle® peptide conjugated to the cytotoxic drug monomethyl auristatin E (MMAE) via a cleavable linker and is currently being investigated in the Phase 1/2 clinical trial Duravelo-1 (NCT04561362), and Phase 2/3 clinical trial, Duravelo-2 (NCT06225596).
- Nectin-4 is an adhesion molecule primarily expressed in normal epithelium and on tumor cells from urothelial, breast, lung and esophageal cancer (3).
- Nectin-4 is targeted by the antibody-drug conjugate enfortumab vedotin (EV, Padcev®) approved by FDA and EC as a monotherapy or in combination with pembrolizumab in locally advanced or metastatic urothelial cancer (4).
- Ocular disorders, or ocular surface toxicities, are reported as treatment-related adverse events of clinical interest in clinical trials evaluating ADCs (5).
- Ocular disorders or ocular surface ADC toxicities are common due to the unique characteristics of the cornea, including rapid proliferation of corneal epithelial cells, high vascularization and expression of Fcγ receptors that bind antibodies (6).
- In the ongoing Duravelo-1 clinical study, grade I/II ocular disorders have been observed in 7% of patients treated with zele at 5 mg/m^2 QW (n=149) (7).
- The EV-301 trial reported that 27% of patients experienced ocular disorders (n=296) (8) and 40% of 384 patients treated with PADCEV as a single agent (PADCEV Safety).

INTRODUCTION

REVERSE TRANSLATIONAL STUDY DESIGN COMPARING EV TO ZELE IN AN EPICORNEAL™ TISSUE MODEL

We hypothesized that zele and EV would show differential biological effects in a human corneal epithelial tissue model, with the short exposure time of zele reducing the risk of corneal tissue damage (7).

Zele and EV effects were determined in Mattek's EpiCorneal model (9), after treatment with parent drug (BTC or ADC) concentrations matching the systemic exposure in human plasma (7, 8) (Figures 1A-D, Table 1).

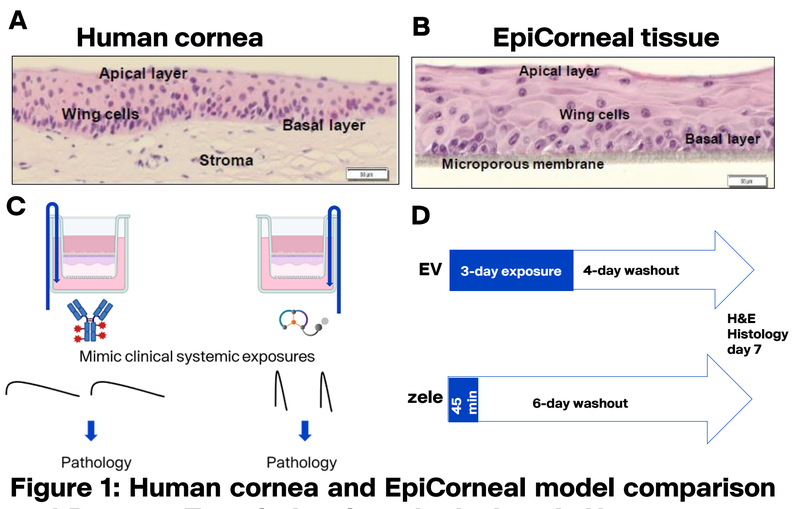


Figure 1: Human cornea and EpiCorneal model comparison and Reverse Translational study design. A. Human cornea H&E staining showing anatomical features B. EpiCorneal tissue (9) C, D. EV at $C_{average}$ and zele at C_{max} treatment based on clinical plasma exposure was added basolaterally to mimic systemic delivery of the pharmaceutical agents. Parent drug top concentration was set to deliver the equivalence of 100 ng/ml total MMAE.

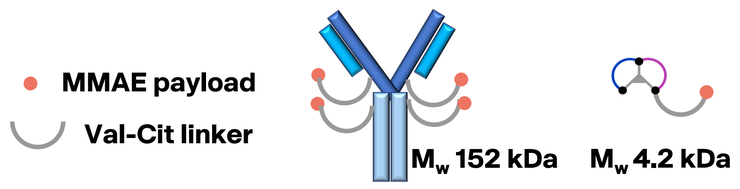
MATERIAL & METHODS

- Nectin-4 protein expression was assessed by immunohistochemistry assay (10) analysis of human eye and Mattek's EpiCorneal model (9).
- The treatment was performed based on pharmacokinetics achieved at the clinical dose [zele C_{max} = 0.6 µg/mL, exposure time 45 minutes; EV $C_{average}$ =5.1 µg/mL, exposure time 3 days]. Parent drug was titrated in half-log steps to determine dose-response and washed out after $t_{1/2}$ exposure time.
- The EpiCorneal tissues were fixed, sectioned and hematoxylin/eosin stained after 7 days and the tissue thickness was quantified in triplicate tissues with 9 measurements per sample.

ACKNOWLEDGEMENTS

MATTEK – EpiCorneal™ model
NEOGENOMICS – Nectin-4 immunohistochemistry
WUXI AppTec – BTC synthesis
EVIDENTIC – clinical ADC material provision

TABLE 1. COMPARISON OF ENFORTUMAB VEDOTIN AND ZELENECTIDE PEVEDOTIN



Property (7,8)	Padcev® enfortumab vedotin (EV)	Zelenectide pevedotin (zele; BT8009)
Targeting modality	Antibody	Constrained, synthetic Bicycle® peptide
Target	Nectin-4	Nectin-4
Molecular weight	152 kDa	4.2 kDa
Toxin-linker conjugate	Val-Cit MMAE	Val-Cit MMAE
Payload ratio	3.8	1
$t_{1/2}$ in plasma	3.6 days	<1 hour
Parent drug in plasma	$C_{average}$ 5.23 µg/ml	C_{max} 0.60 µg/ml
Total concentration MMAE	$C_{average}$ 102 ng/ml	C_{max} 107 ng/ml
Parent drug concentrations adjusted to deliver equivalence of total MMAE	100 ng/mL total MMAE ↔ 5.1 µg/mL EV	100 ng/mL total MMAE ↔ 0.6 µg/mL zele

RESULTS: NECTIN-4 EXPRESSION IN HUMAN CORNEA AND EPICORNEAL TISSUE MODEL

- Nectin-4 is a cell adhesion molecule that is particularly abundant in epithelial tissues, where it plays a role in adherens junctions. Immunohistochemistry (IHC) analysis of human eye tissue demonstrated expression of Nectin-4 predominantly in the stratified epithelium of the cornea (Figure 2).
- Characterization of Mattek's EpiCorneal tissue confirmed expression of Nectin-4 (Figure 3A) and the H-score quantification showed that Nectin-4 is mainly present in the cellular membrane and is not significantly altered by treatment conditions (Figure 3B).

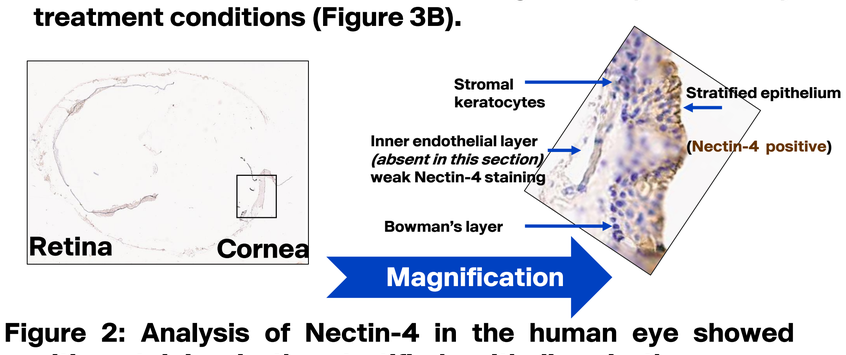


Figure 2: Analysis of Nectin-4 in the human eye showed positive staining in the stratified epithelium in the cornea. Bicycle's Nectin-4 IHC assay (10) was implemented by Neogenomics to stain a cross-section of human eye tissue.

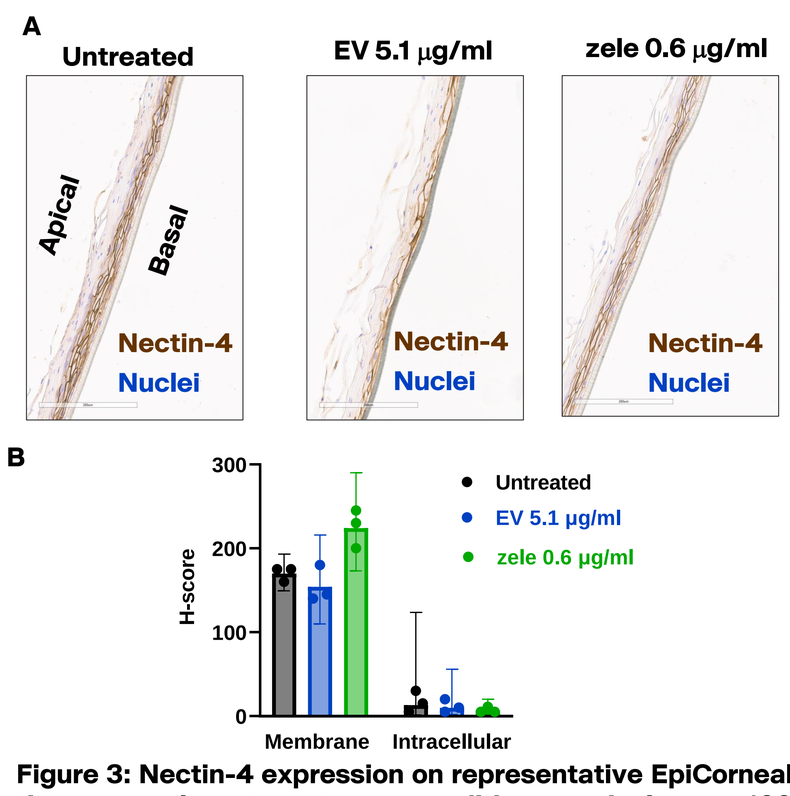


Figure 3: Nectin-4 expression on representative EpiCorneal tissue samples at treatment conditions equivalent to 100 ng/ml total MMAE. A. Representative IHC images from untreated, EV and zele-treated EpiCorneal tissues using Bicycle's Nectin-4 IHC protocol (10). B. H-score mean +/- st dev was determined for membrane and intracellular Nectin-4 expression n=3 tissues per condition.

RESULTS: REDUCTION IN EPICORNEAL TISSUE THICKNESS AFTER EV TREATMENT

- Histological analysis of EpiCorneal tissue thickness showed untreated control tissue measuring 35±2 micrometers (µm) with $C_{average}$ 5.1 µg/mL EV-treated tissue measuring 24±3 µm, and C_{max} 0.6 µg/mL zele-treated tissue 35±3 µm. Significant tissue thinning was caused by $C_{average}$ EV treatment with no effect of zele at plasma equivalent C_{max} exposure.

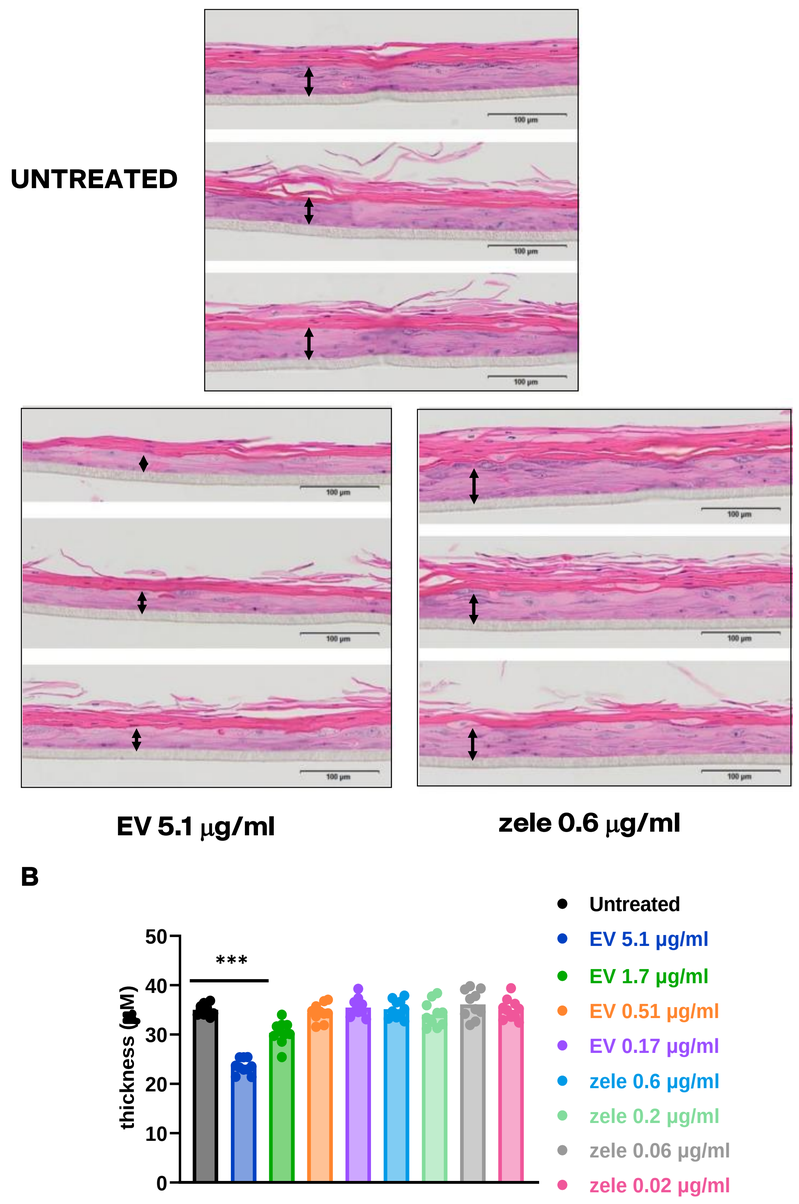


Figure 4: Tissue thickness in untreated controls, EV-treated and zele-treated EpiCorneal tissues. A. Triplicate EpiCorneal tissue sample H&E staining of 5.1 µg/ml EV $C_{average}$ treatment and 0.6 µg/ml zele C_{max} treatment (Figure 1D). Scale bar indicating 100 µM, arrow illustrating EpiCorneal tissue thickness B. Quantification of tissue thickness across Dunnett's multiple comparisons statistical test showed p<0.0001 comparing the untreated tissues to EV-treated tissues at $C_{average}$ 5.1 µg/mL.

CONCLUSIONS

- Nectin-4 expression was demonstrated in corneal stratified epithelium of human eye, supporting the relevance of using in vitro generated human cornea tissue model to explore the risk of on-target mediated toxicity.
- We utilised a reverse translation study design, at clinically relevant human plasma concentrations and exposure times of EV at $C_{average}$ and zele at C_{max} to enable comparison of tissue damage in the EpiCorneal model.
- EV $C_{average}$ treatment induced more severe detrimental effects on EpiCorneal tissue thickness compared to zele C_{max} treatment as demonstrated by significant thinning at EV clinical dose equivalent to 100 ng/mL total MMAE.
- In conclusion, the low incidence of ocular disorders in zele-treated patients is recapitulated in the EpiCorneal model.

SAFETY DEFINITIONS AND ABBREVIATIONS

- For zelenectide pevedotin AEs of interest, the preferred terms (PT) were defined by Standardised MedDRA®version 27.0 and severity and grade was assigned by Investigator per National Cancer Institute Common Terminology Criteria for Adverse Events v5.0
- Treatment-emergent Adverse Events (≥ 10% of Subjects in the Enfortumab Vedotin 1.25 mg/kg Group) by SOC and Preferred Term (Safety Analysis Set). Study EV-301 Assessment report EMA/249357/2022

- Abbreviations: ADCs, antibody drug conjugates; BTC®, Bicycle Toxin Conjugate, $C_{average}$, average concentration; C_{max}, maximal concentration; EV, enfortumab vedotin; H&E, hematoxylin and eosin; MMAE, monomethyl auristatin E; QW, weekly; PK, pharmacokinetics; zele, zelenectide pevedotin

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Bicycle Therapeutics, Inc.
35 Cambridgepark Drive, Suite 350
Cambridge, MA 02140
USA
T. +1 617-945-8155

BicycleTx Limited
Portway Building
Granta Park, Cambridge
CB21 6GS, UK
T. +44 (0)1223 261503

Company number 11036101.
Registered in England.

bicycletherapeutics.com