



# Preclinical assessment of nuzefatide pevedotin (nuzefa; formerly BT5528) anti-tumor activity in cell-line-derived xenograft models of head and neck squamous cell carcinoma

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# Disclosure Information

Gavin Bennett is an employee of BicycleTx Limited and owns equity in the parent company, Bicycle Therapeutics Limited.

# Nuzefa Is a Bicycle<sup>®</sup> Drug Conjugate Targeting EphA2

## Nuzefa: Bicycle<sup>®</sup> Drug Conjugate (BDC<sup>®</sup>)

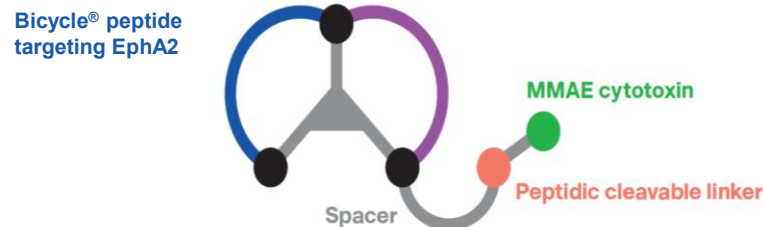
- Highly selective erythropoietin-producing hepatocellular receptor A2 (EphA2)–targeting bicyclic peptide conjugated to monomethyl auristatin E (MMAE) via a stable valine-citrulline cleavable linker<sup>1-3</sup>

Nuzefa (formerly BT5528) is currently under investigation in patients with solid tumors (NCT04180371,<sup>4</sup> NCT07450859<sup>5</sup>)

In a Phase 1 first-in-human study, nuzefa was well tolerated and demonstrated favorable preliminary anti-tumor activity as monotherapy, particularly in metastatic urothelial carcinoma<sup>2</sup>

The preliminary safety profile of nuzefa differentiates it from hematologic toxicity associated with other EphA2-targeting antibody-drug conjugates<sup>1-3,6</sup>

## Nuzefa Structure and Characteristics



<b>Molecular weight</b>	4.4 kDa <sup>1</sup>
<b>Half-life</b>	<1 hr <sup>6</sup>
<b>Affinity for EphA2 (K<sub>D</sub>)</b>	1.9 nM <sup>1,a</sup>
<b>Specificity of binder</b>	Binds to a single protein in over 5500 surface-expressed targets <sup>7,b</sup>

<sup>a</sup>As assessed by fluorescence polarization. <sup>1</sup> <sup>b</sup>As assessed by Retrogenix membrane array.<sup>7</sup>

BDC<sup>®</sup>, Bicycle<sup>®</sup> drug conjugate; EphA2, erythropoietin-producing hepatocellular receptor A2; hr, hour; K<sub>D</sub>, dissociation constant; kDa, kilodaltons; MMAE, monomethyl auristatin E; nM, nanomolar; nuzefa, nuzefatide pervedotin.

1. Bennett G, et al. *Mol Cancer Ther*. 2020;19(7):1385–1394. 2. Bashir B, et al. *J Clin Oncol*. 2024;42(29):3443–3452. 3. Bennett G, et al. *J Transl Med*. 2026;24(1):322. 4. Clinicaltrials.gov. <https://clinicaltrials.gov/study/NCT04180371>. Accessed March 2026. 5. ClinicalTrials.gov. <https://clinicaltrials.gov/study/NCT07450859>. Accessed March 2026. 6. Bader J, et al. *J Clin Oncol*. 2024;42(Suppl 16):Abstract 3088. 7. BicycleTx Ltd unpublished data.

# Unmet Need for Treatments Targeting EphA2-expressing Tumors

EphA2 is a receptor tyrosine kinase critical for cellular function and tissue development<sup>1</sup>

EphA2 is highly expressed in a range of solid tumors,<sup>2</sup> and its expression correlates with higher grade, later-stage disease,<sup>3,4</sup> and poor prognosis<sup>2</sup>

Earlier EphA2-targeted therapies have been associated with substantial toxicities such as hemorrhage and peripheral neuropathy that have limited efficacy analysis<sup>5-9</sup>

There is a high unmet need for effective therapies for patients with EphA2-expressing tumors,<sup>10</sup> including head and neck squamous cell carcinoma (HNSCC)<sup>3</sup>

EphA2, erythropoietin-producing hepatocellular receptor A2; HNSCC, head and neck squamous cell carcinoma.

1. Park JE, et al. *Genes*. 2013;4(3):334–357. 2. Nehal M, et al. *Mol Biol Rep*. 2024;51(1):337. 3. Liu Y, et al. *J Cancer Res Clin Oncol*. 2011;137(5):761–769. 4. Abraham S, et al. *Clin Cancer Res*. 2006;12(2):353–360. 5. Bennett G, et al. *J Transl Med*. 2026;24(1):322. 6. Shitara K, et al. *J Immunother Cancer*. 2019;7(1):219. 7. Annunziata CM, et al. *Invest New Drugs*. 2013;31(1):77–84. 8. Merrimack Pharmaceuticals, Inc. Press release 4th April 2019. <https://www.prnewswire.com/news-releases/merrimack-discontinues-development-of-mm-310-300825018.html>. 9. ClinicalTrials.gov. <https://clinicaltrials.gov/study/NCT03076372>. Accessed February 2026. 10. Tandon M, et al. *Expert Opin Ther Targets*. 2011;15(1):31–51.

# Patients With HNSCC Can Have High EphA2 Tumor Expression and Poor Prognosis

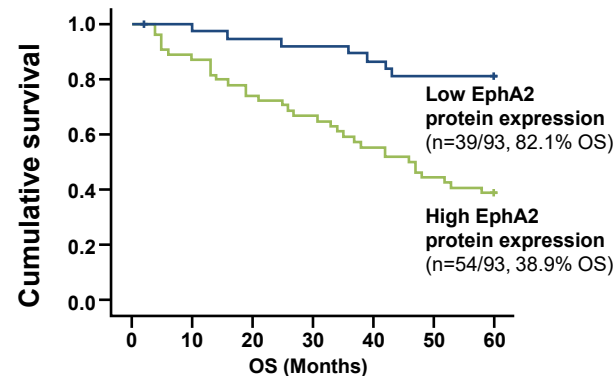
High EphA2 expression was observed in HNSCC patient tumor samples and cell lines and was associated with poorer overall survival<sup>1</sup>

## Immunohistochemistry (IHC) of EphA2 in human HNSCC tissues

EphA2 protein expression (N=98)			
Negative control	Low, n (%)	Moderate, n (%)	High, n (%)
	17 (17.3%)	27 (27.6%)	54 (55.1%)
<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>
<b>E</b>	<b>F</b>	<b>G</b>	<b>H</b>

Immunohistochemistry of EphA2 in human HNSCC tissues. **A, E** Negative control of EphA2 in HNSCC tumor tissues (primary antibody replaced with normal goat serum). **B, F** Negative/weak expression of EphA2 in HNSCC tumor tissues (Scored 3). **C, G** Moderate expression of EphA2 in HNSCC tumor specimens (Scored 6). **D, H** Strong expression of EphA2 in HNSCC tumor specimens (Scored 9) (original magnification  $\times 100$  in **A–D**;  $\times 400$  in **E–H**).

## Overall survival (OS) curve<sup>a</sup>



<sup>a</sup>Constructed by the Kaplan-Meier survival method; grouped by EphA2 expression (low vs high). OS was calculated from the day of surgery to the date of death or tumor relapse. Mean follow-up time was 45 months (range, 2 to 60 months).

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EphA2, erythropoietin-producing hepatocellular receptor A2; HNSCC, head and neck squamous cell carcinoma; IHC, immunohistochemistry; OS, overall survival.

1. Liu Y, et al. *J Cancer Res Clin Oncol.* 2011;137(5):761–769.

# Objective

To evaluate anti-tumor activity of nuzefa in murine cell line-derived xenograft (CDX) models of HNSCC

# Methods: Preclinical Assessment of Nuzefa Activity

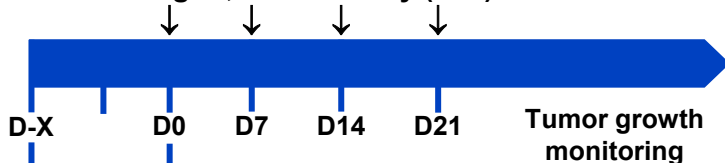
- Three mouse models were generated by subcutaneous (SC) inoculation of CDX HNSCC tumor cells into the right abdominal flank
- Cell lines were selected from different tissue sources with a range of EphA2 expression levels for activity analysis



**MODEL**

Fadu – Balb/c nude (F)  
SCC-9 – NOD SCID (F)  
Cal27 – Balb/c nude (F)

**Dosing IV, once weekly (QW)×4**



**Inoculation**  
Day -4 (SCC-9)  
Day -17 (Fadu)  
Day -21 (Cal27)

At dosing initiation, mean tumor volume ~160 mm<sup>3</sup>

- **Dosing:**
  - Tumor-bearing mice were treated QW by IV over 4 weeks
- **Four treatment groups: n=6 per group**
  - Vehicle
  - Nuzefa: 1, 3, and 5 mg/kg
- **EphA2 expression assessed in tumor cells by:**
  - Quantitative fluorescence-activated cell sorting (FACS)
  - Immunohistochemistry (H-score) on tumor xenografts
- **Tumor evaluation:**
  - Growth monitored by caliper
  - Volume calculated as  $[\text{width}^2 \times \text{length}]/2$
- **Average tumor growth inhibition (TGI):**
  - Calculated for each treatment group per model for the last timepoint with a complete dataset up to 28 days post treatment initiation

# Results: EphA2 Expression

Average EphA2 expression in SCC-9, Fadu, and Cal27 was comparable to levels observed in previous preclinical work in CDX and PDX models

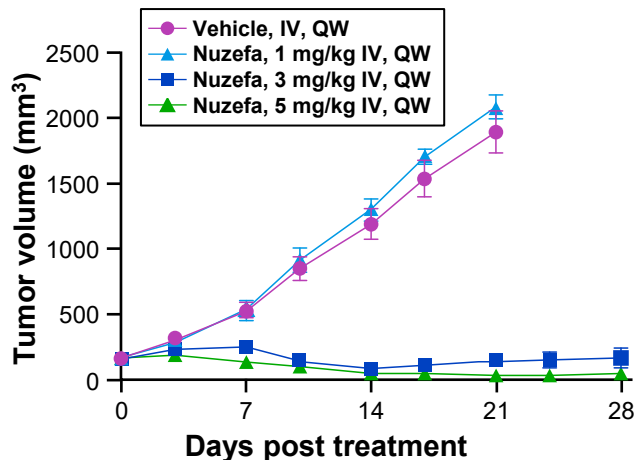
EphA2 expression in HNSCC cell lines did not consistently correlate with xenograft membrane H-score

Cell line	Source	EphA2 expression <sup>a</sup>	H-score <sup>b</sup>
SCC-9	tongue	29,000	50 (high)
Fadu	hypopharynx	11,500	20 (medium)
Cal27	tongue	18,500	15 (low)

<sup>a</sup>Measured by FACS as the average number of receptors per cell. <sup>b</sup>H-score based on membrane only quantification.

# Results: Treatment With Nuzefa Demonstrated Substantial Anti-tumor Activity in the SCC-9 Tumor

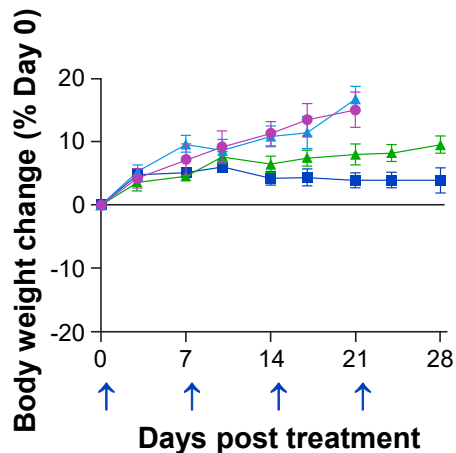
## Treatment effects on tumor volume



### SCC-9 Day 21

- Nuzefa 3 mg/kg: **TGI 101%** ( $P < 0.0001$ )
- ▲ Nuzefa 5 mg/kg: **TGI 107%** ( $P < 0.0001$ )

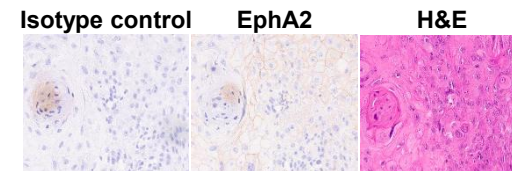
## Treatment effects on body weight



↑ = Treatment administered

## SCC-9 CDX model characteristics

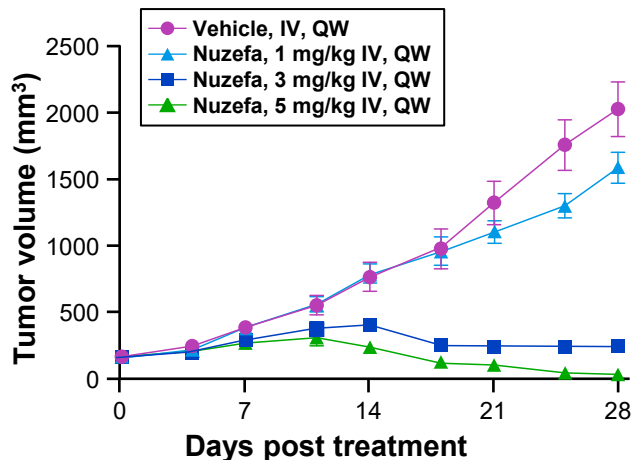
- **Mouse:** female, NOD SCID
- **Tissue:** tongue
- **Disease:** squamous cell carcinoma
- ▶ **EphA2 expression, based on membrane H-score: high**



29,000 antibody binding capacity (ABC)  
 H-score=50  
 TPS=50

# Results: Treatment With Nuzefa Demonstrated Substantial Anti-tumor Activity in the Fadu Tumor

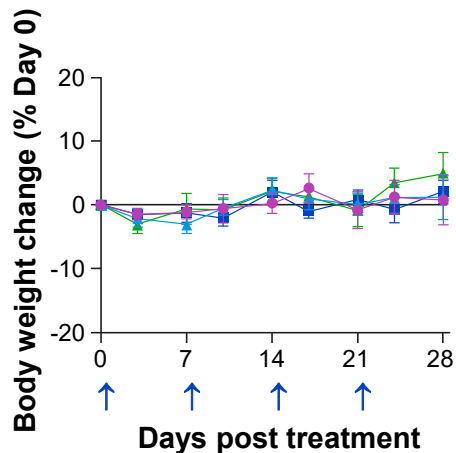
## Treatment effects on tumor volume



### Fadu Day 25

- Nuzefa 3 mg/kg: **TGI 95%** ( $P < 0.0001$ )
- ▲ Nuzefa 5 mg/kg: **TGI 107%** ( $P < 0.0001$ )

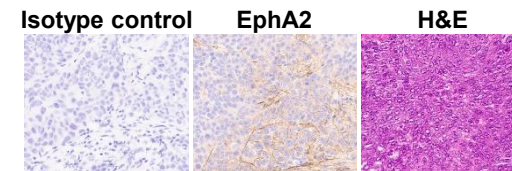
## Treatment effects on body weight



↑ = Treatment administered

## Fadu CDX model characteristics

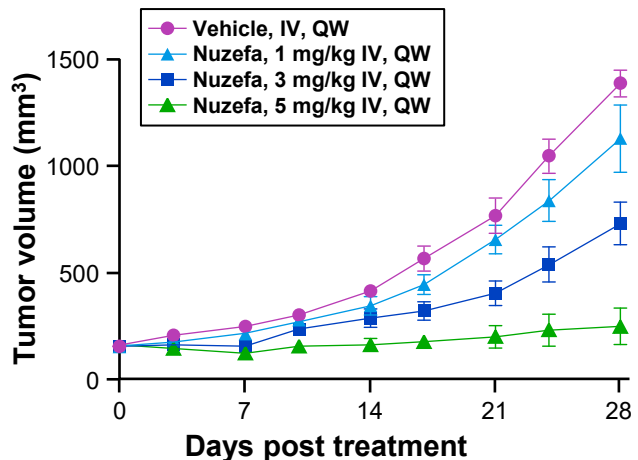
- **Mouse:** female, Balb/c nude
- **Tissue:** hypopharynx
- **Disease:** squamous cell carcinoma
- ▶ **EphA2 expression, based on membrane H-score: medium**



11,500 ABC  
 H-score=20  
 TPS=20

# Results: Treatment With Nuzefa Demonstrated Substantial Anti-tumor Activity in the Cal27 Tumor

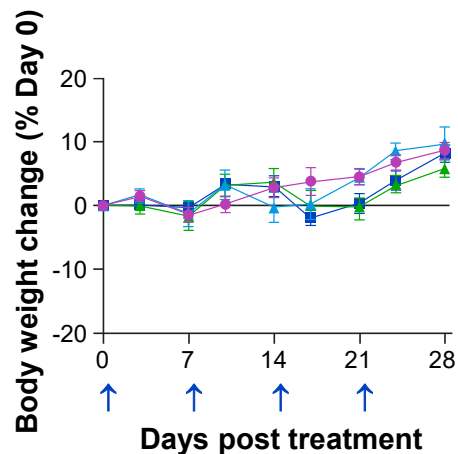
## Treatment effects on tumor volume



### Cal27 Day 28

- Nuzefa 3 mg/kg: **TGI 53%** ( $P < 0.001$ )
- ▲ Nuzefa 5 mg/kg: **TGI 92%** ( $P < 0.0001$ )

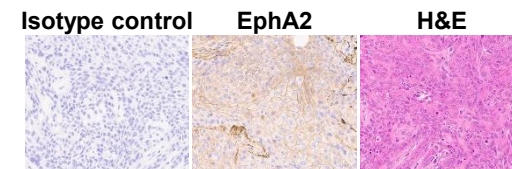
## Treatment effects on body weight



↑ = Treatment administered

## Cal27 CDX model characteristics

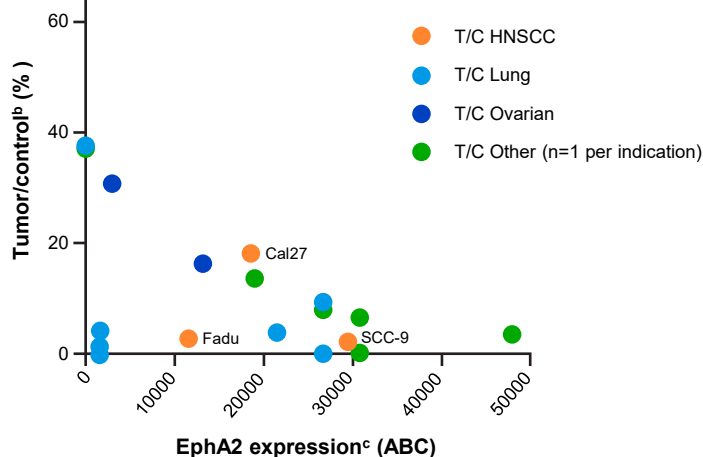
- **Mouse:** female, Balb/c nude
- **Tissue:** tongue, epithelial
- **Disease:** squamous cell carcinoma
- ▶ **EphA2 expression, based on membrane H-score: low**



18,500 ABC  
 H-score=15  
 TPS=15

# Results: Relationship Between EphA2 Expression and Nuzefa Anti-tumor Activity in CDX and PDX

Expression vs nuzefa anti-tumor activity analysis across preclinical models<sup>a</sup>



<sup>a</sup>Nuzefa dosed at 3 mg/kg IV, QW.

<sup>b</sup>Tumor/control (T/C) ratio calculated at last data point before control group terminated.

<sup>c</sup>EphA2 expression measured by FACS (antigen binding sites per cell).

- Nuzefa demonstrates robust anti-tumor activity in HNSCC models, consistent with its performance in other preclinical models previously tested<sup>1,2</sup>
- Cal27, which had the lowest membrane H-score, showed decreased sensitivity to treatment compared with SCC-9 and Fadu

ABC, antibody binding capacity; CDX, cell line-derived xenograft; EphA2, erythropoietin-producing hepatocellular receptor A2; FACS, fluorescence-activated cell sorting; Fadu, faryngeal/pharyngeal-ductal; H-score, histochemical score; HNSCC, head and neck squamous cell carcinoma; IV, intravenous; PDX, patient-derived xenograft; QW, once weekly; SCC, squamous cell carcinoma; T/C, tumor control ratio.

# Conclusions

Nuzefa demonstrated potent preclinical anti-tumor activity in EphA2-expressing CDX models of HNSCC at clinically relevant doses

As expected, no tolerability issues were observed during treatment with nuzefa, based on the evaluation of percent body weight changes over time

These data support further evaluation of therapeutically targeting EphA2 in HNSCC

**Future directions:** Nuzefa is currently being investigated in both Phase 1/2 and Phase 2 trials in patients with advanced solid tumor types that are associated with EphA2 expression (NCT04180371,<sup>1</sup> NCT07450859<sup>2</sup>)

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- ▶ #CT063      Poster      Monday April 20, 2026
- ▶ #4518        Poster      Tuesday April 21, 2026