

# Preclinical assessment of nuzefatide pevedotin (nuzefa; formerly BT5528) anti-tumor activity in patient-derived xenograft models of pancreatic ductal adenocarcinoma

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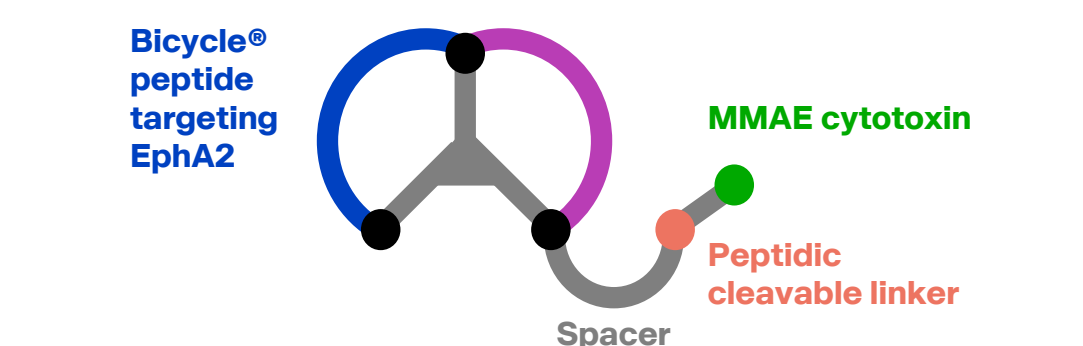
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## BACKGROUND

- Erythropoietin-producing hepatocellular receptor A2 (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 toxicity that has limited efficacy analysis<sup>5-8</sup>
- There is high unmet need for new treatment options for patients with EphA2-expressing tumors,<sup>9</sup> including pancreatic cancer, which has one of the highest EphA2 expression levels across solid tumors<sup>10</sup>
- Bicycle® molecules are an innovative therapeutic class under clinical evaluation that offers the manufacturing and pharmacokinetic properties of a small molecule with the high binding specificity of an antibody,<sup>11-14</sup> making them ideally suited for the targeted delivery of a range of payloads, such as cytotoxins, to solid tumors
- Nuzefatide pevedotin (nuzefa; formerly BT5528) is a Bicycle® Drug Conjugate (BDC®), comprising a highly selective EphA2-targeting bicyclic peptide conjugated to the cytotoxin monomethyl auristatin E (MMAE) via a stable valine-citrulline cleavable linker (Figure 1)<sup>11,12</sup>
- Nuzefa has low molecular weight (4.4 kDa), enabling rapid and efficient delivery of the BDC® to the tumor and subsequent payload release, with minimal systemic exposure to the conjugate<sup>11</sup>

## FIGURE 1. SCHEMATIC AND CHARACTERISTICS OF NUZEFA<sup>11,13</sup>



<b>Molecular weight</b>	4.4 kDa <sup>11</sup>
<b>Half-life</b>	<1 hr <sup>15</sup>
<b>Affinity for EphA2 (K<sub>d</sub>)</b>	1.9 nM <sup>11,a</sup>
<b>Specificity of binder</b>	Binds to a single protein in over 5500 surface-expressed targets <sup>16,b</sup>

<sup>a</sup>As assessed by fluorescence polarization<sup>11</sup> <sup>b</sup>As assessed by Retrogenix membrane array<sup>16</sup>  
EphA2, erythropoietin-producing hepatocellular receptor A2; K<sub>d</sub>, dissociation constant; kDa, kilodalton; MMAE, monomethyl auristatin E; nuzefa, nuzefatide pevedotin.

## OBJECTIVE

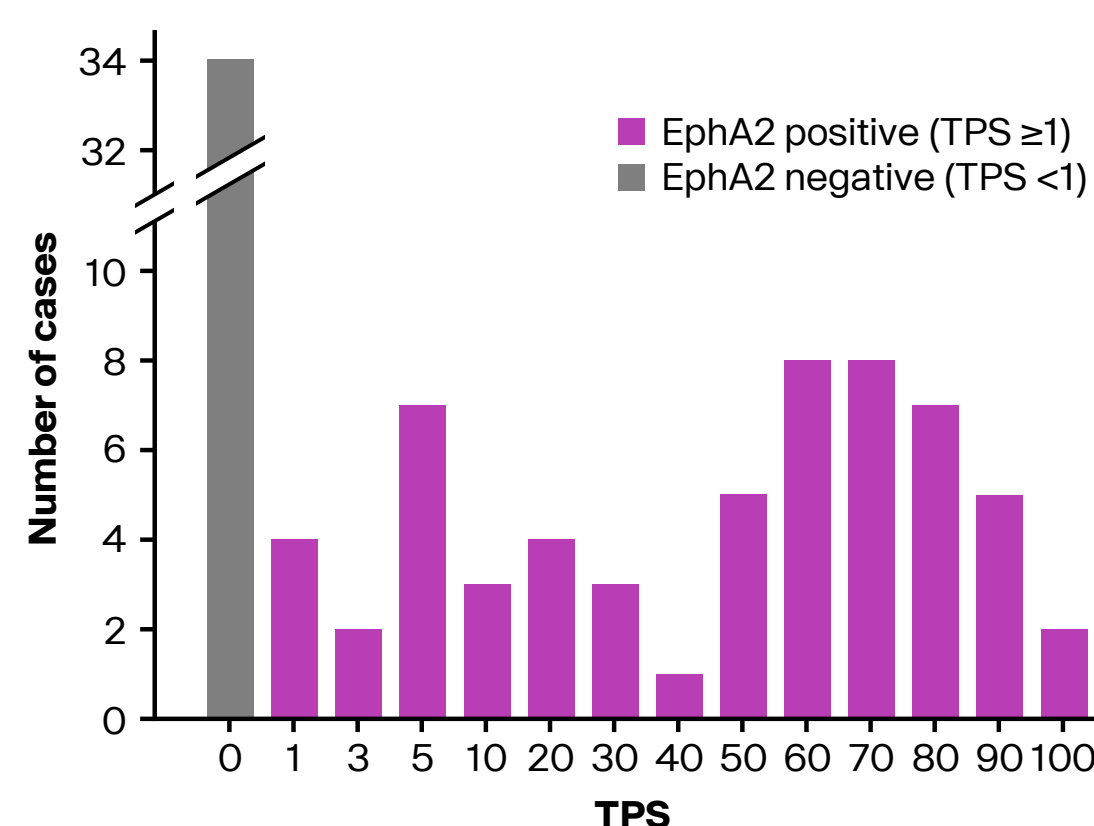
- To evaluate EphA2 expression and anti-tumor activity of nuzefa in murine patient-derived xenograft (PDX) models of pancreatic cancer

## METHODS

- Cohorts of female NOD SCID gamma (NSG) mice were used to generate 16 pancreatic PDX models by subcutaneous implantation of patient-derived pancreatic tumors into the left abdominal flank
- Paraffin-embedded tumor samples from vehicle-treated mice were used to assess EphA2 expression by immunohistochemistry (IHC)
  - EphA2 IHC was performed using rabbit monoclonal antibody CST 6997 (Cell Signaling Technology) that detects the intracellular domain of EphA2
- Tumor proportion score (TPS) was calculated as the number of membrane EphA2+ cells/total number of viable tumor cells × 100
  - TPS ≥1% was used to determine positivity
- Tumor-bearing mice were treated once weekly for 4 weeks with either vehicle control or nuzefa (3 mg/kg IV)
- Tumor growth was monitored by caliper measurements, and tumor volume was calculated as (width)<sup>2</sup> × length/2
- Tumor growth inhibition (TGI) was estimated for each treatment group per model using the average response across replicates after 2–4 weeks

## RESULTS

### FIGURE 2: MOST SAMPLES IN HUMAN PANCREATIC TUMOR MICROARRAY PA1921C WERE EPHA2 POSITIVE (TPS ≥1)



EphA2, erythropoietin-producing hepatocellular receptor A2; TPS, tumor proportion score.

- Most samples in human pancreatic tumor microarray PA1921c were EphA2 positive (TPS ≥1) (Figure 2)
- Among 93 cases (179 cores) in a pancreatic cancer tissue microarray (Figure 2):
  - 34 (37%) were EphA2 negative
  - 59 (63%) were EphA2 positive

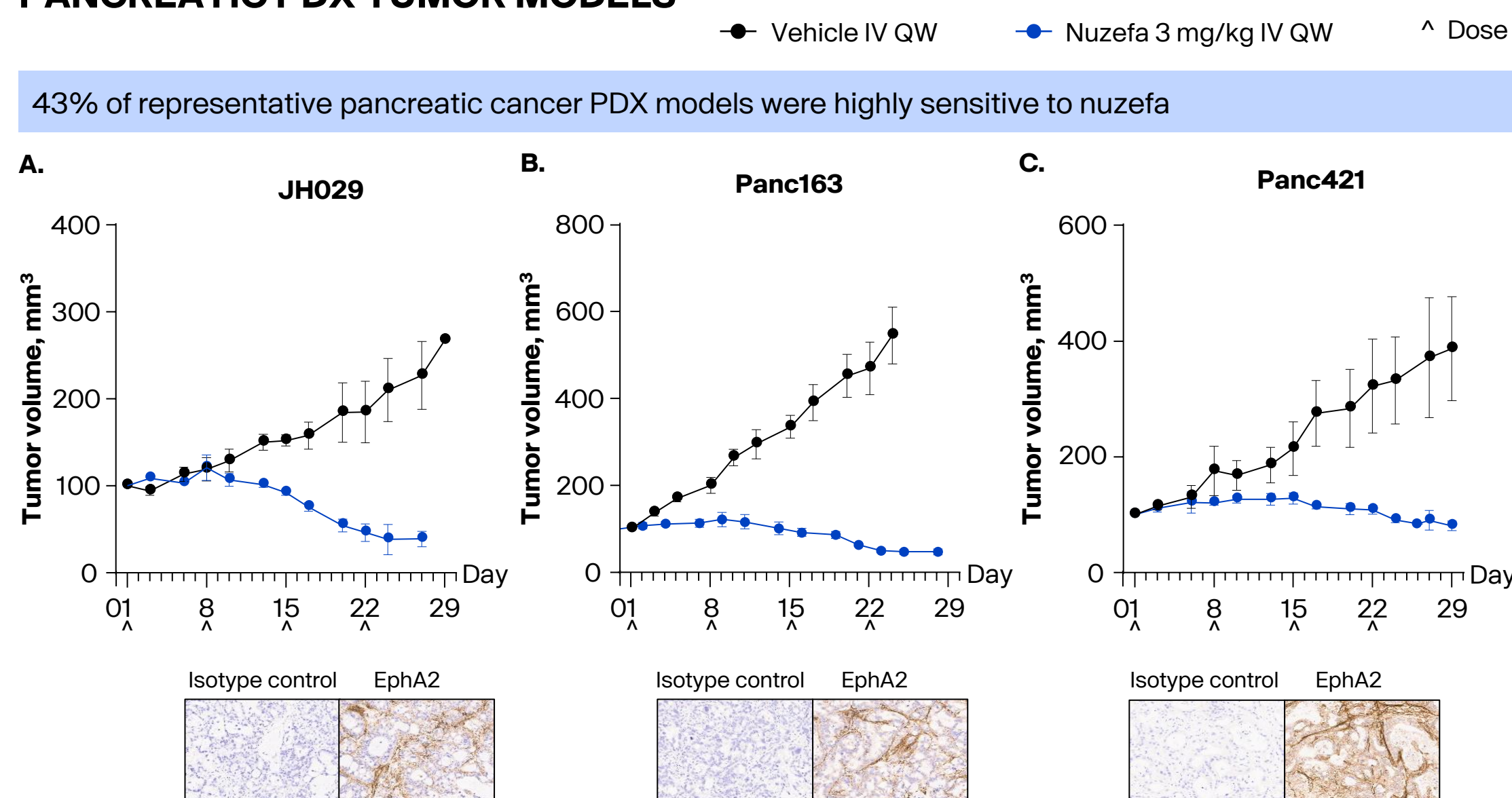
### TABLE 1. SENSITIVITY OF PANCREATIC PDX MODELS TO NUZEFA

PDX ID	TPS Score, %	Nuzefa TGI, %	Desmoplasia Grade
JH029	20	157	Mature
Panc163	30	114	Intermediate
Panc286	55	111	Mature
Panc421	90	110	Immature
Panc033	50	103	N/A
Panc030	50	100	Mature
Panc008	67	91	Mature
Panc281	80	75	Intermediate
JH033	18	70	Mature
JH051	1	52	Immature
Panc094	20	50	Immature
Panc287	60	31	Intermediate
JH094	67	22	Immature
Panc028	80	5	Mature

N/A, not applicable; PDX, patient-derived xenograft; TGI, tumor growth inhibition; TPS, tumor proportion score.

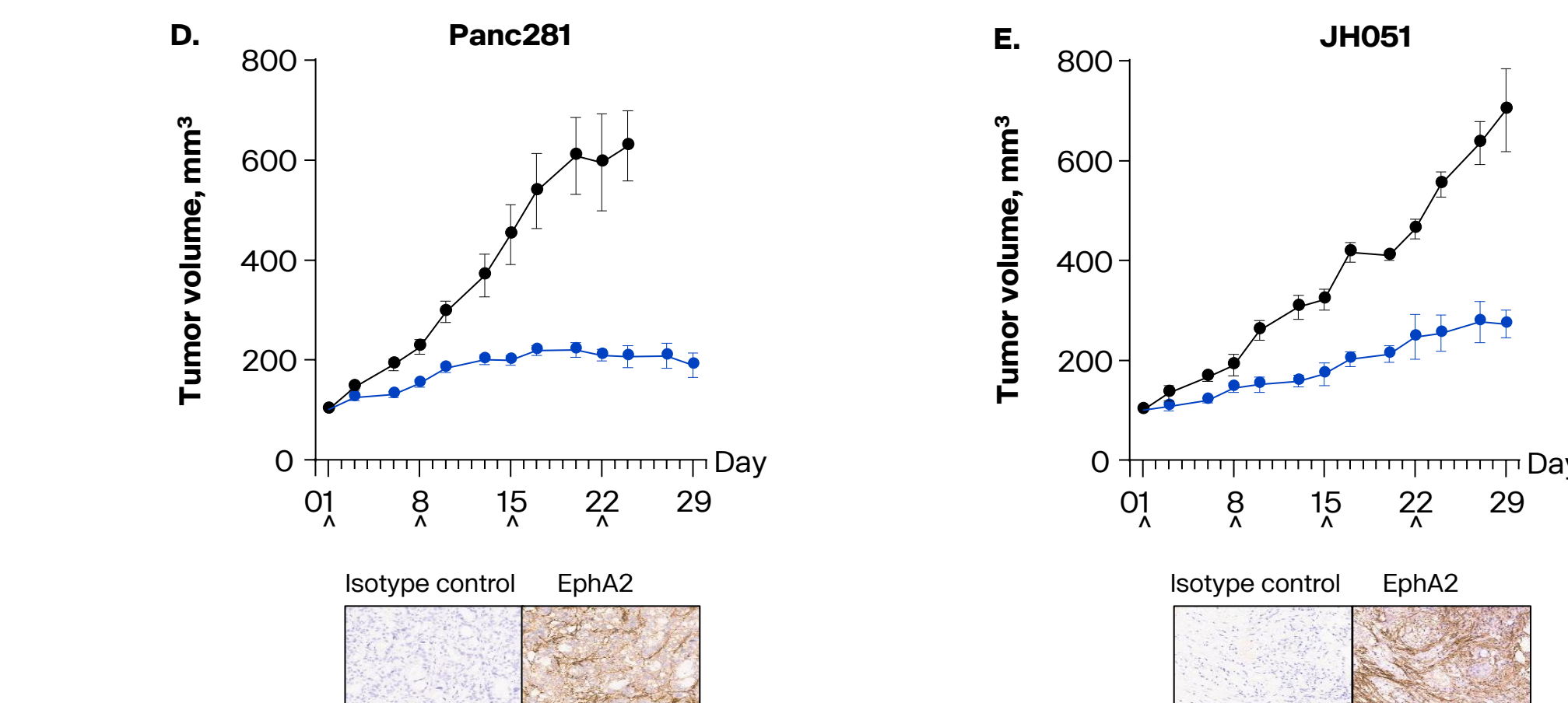
- Of 16 pancreatic PDX models tested by IHC, all 16 were positive for EphA2 membrane staining (TPS ≥1%), including models JH029 (20%), Panc163 (30%), and Panc421 (90%) (Table 1)
- Fourteen of 16 pancreatic PDX models were assessed for anti-tumor activity
- Six of 14 models showed high sensitivity to nuzefa (TGI ≥100%), including JH029 (157%), Panc163 (114%), and Panc421 (110%), with most models showing some level of sensitivity to nuzefa (only 3/14 had TGI <50%) (Table 1 and Figure 3)

### FIGURE 3. NUZEFA DEMONSTRATED ANTI-TUMOR ACTIVITY IN PRECLINICAL PANCREATIC PDX TUMOR MODELS

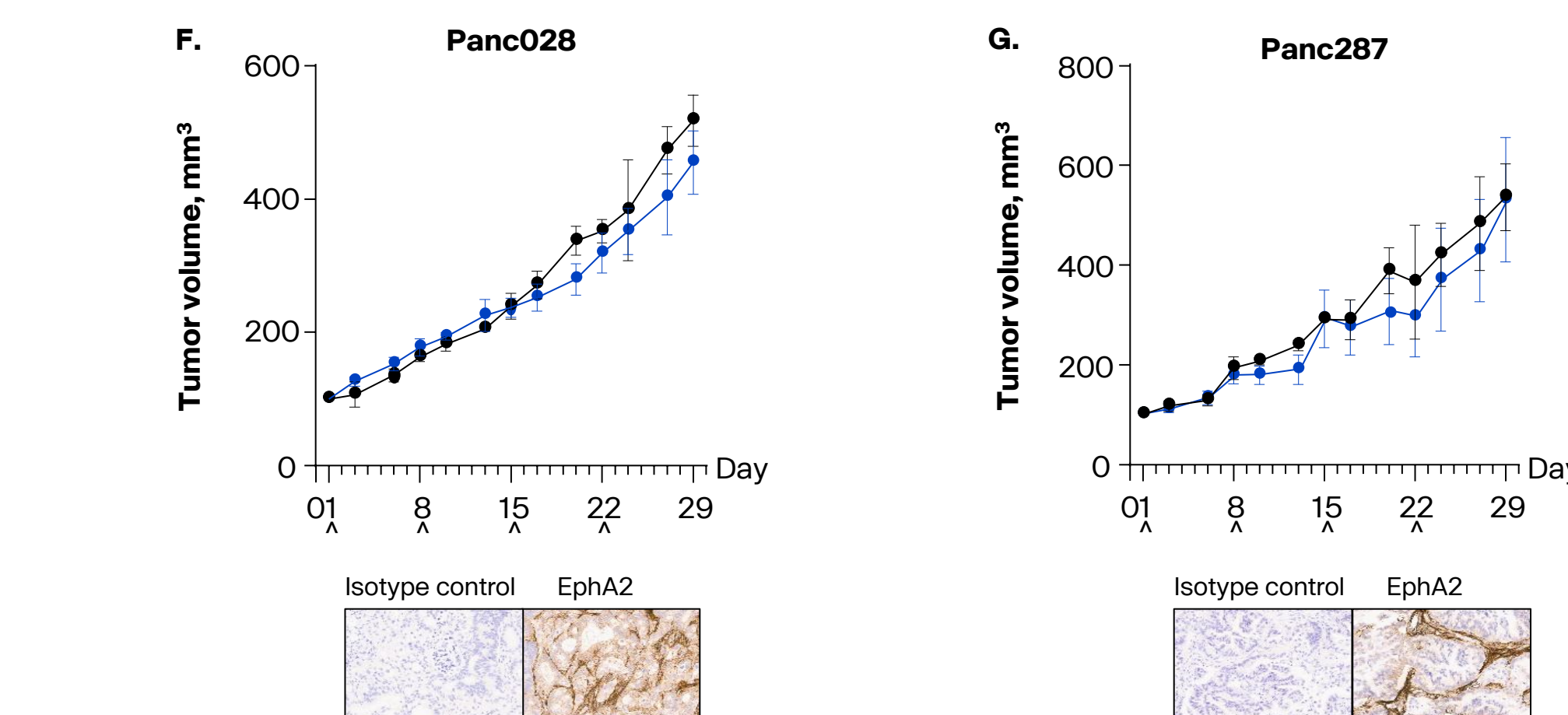


43% of representative pancreatic cancer PDX models were highly sensitive to nuzefa

28.5% of representative pancreatic cancer PDX models had partial sensitivity to nuzefa



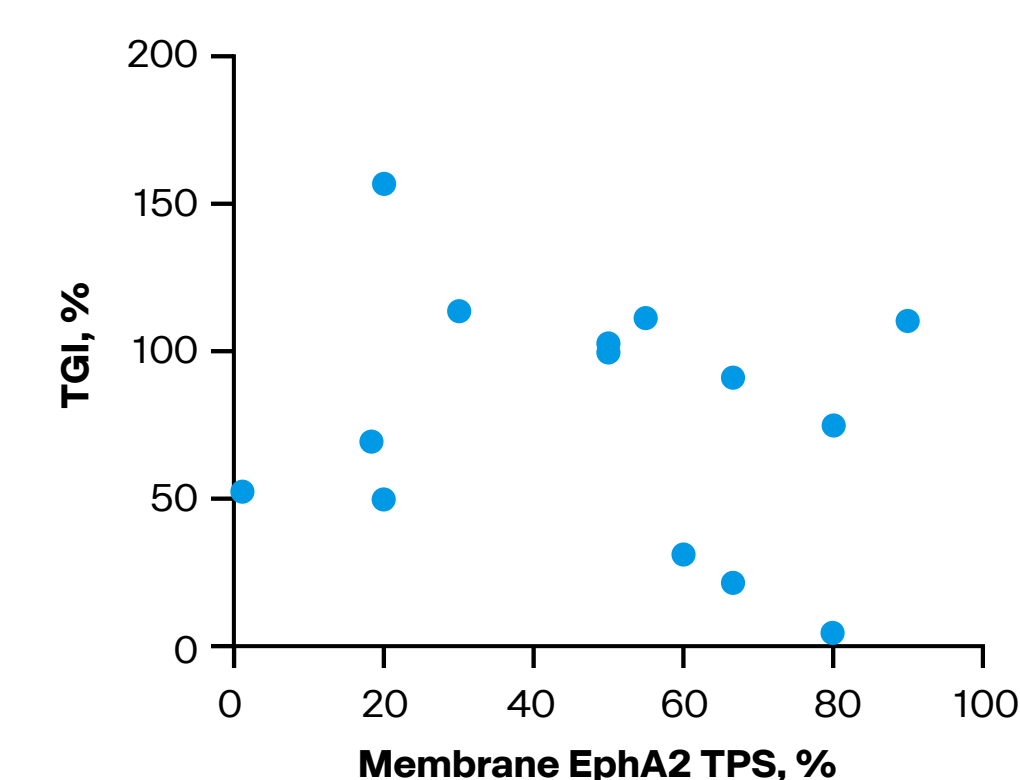
28.5% of representative pancreatic cancer PDX models had no sensitivity to nuzefa



Tumor growth expressed as average tumor volume ± SEM. D1, Day 1; EphA2, erythropoietin-producing hepatocellular receptor A2; IV, intravenous; QW, once weekly; SEM, standard error of the mean.

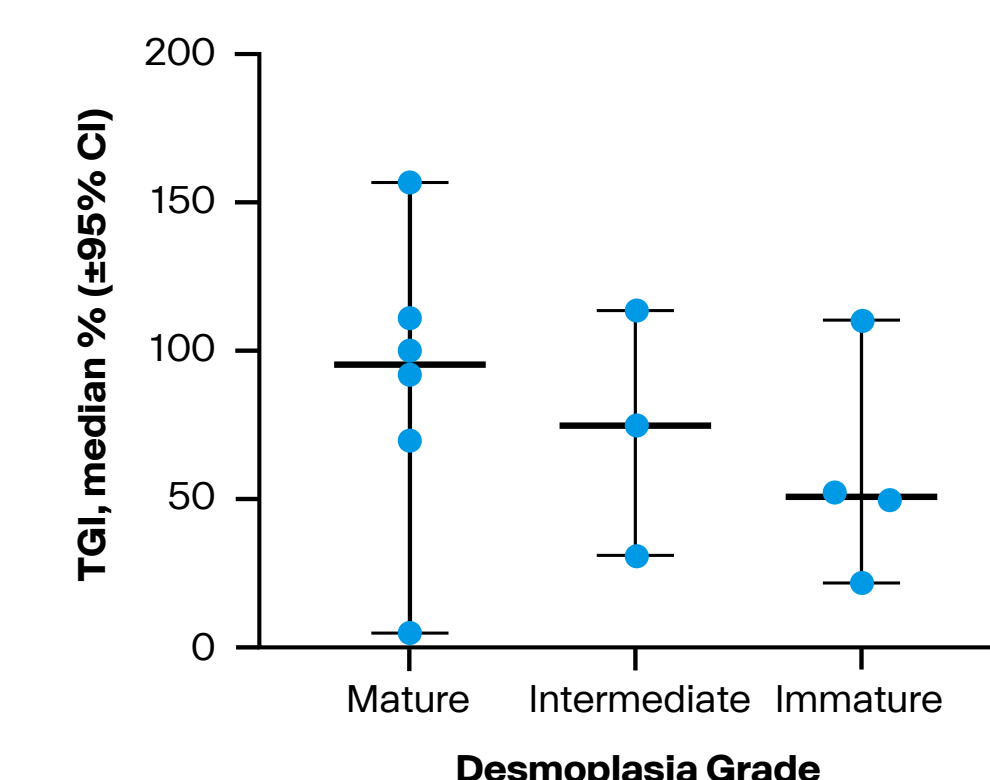
- Nuzefa activity did not significantly correlate with EphA2 expression or desmoplasia grade (TPS or histochemical score [H-score]) (Figures 4 and 5)
- In 14 pancreatic PDX models, TGI following treatment with nuzefa was not correlated with membrane EphA2 TPS (Pearson correlation R<sup>2</sup>=0.033, P=0.5349) (Figure 4)
- Because of the wide range of TGI in the PDX models with mature desmoplasia, no clear effect of the grade of desmoplasia on the TGI was identified (Table 1 and Figure 5)

### FIGURE 4. NO INCREASED NUZEFA ACTIVITY WITH INCREASED MEMBRANE EPHA2 EXPRESSION



There was no correlation between membrane EphA2 TPS in 14 pancreatic PDX models and TGI following treatment with nuzefa (Pearson correlation R<sup>2</sup>=0.033, P=0.5349). If multiple samples were analyzed for H-score, their mean value was used for analysis. EphA2, erythropoietin-producing hepatocellular receptor A2; PDX, patient-derived xenograft; TGI, tumor growth inhibition; TPS, tumor proportion score.

### FIGURE 5. DESMOPLASIA GRADE HAD NO CLEAR EFFECT ON ANTI-TUMOR ACTIVITY OF NUZEFA



Nuzefa TGI across 13 pancreatic PDX models according to their grade of desmoplasia. Grade of desmoplasia does not apply to Panc033 since it is squamous cell carcinoma. CI, confidence interval; PDX, patient-derived xenograft; TGI, tumor growth inhibition.

## CONCLUSIONS

- Expression of EphA2 was found in all preclinical pancreatic PDX models
- Most models (71.5%) were sensitive to nuzefa treatment, and this was not affected by the degree of desmoplasia or membrane EphA2 TPS

- These results may reflect a lack of truly EphA2-negative models in this analysis
- These data support the potential for nuzefa to offer a novel option for the treatment of pancreatic cancer

## FUTURE DIRECTIONS

- Nuzefa is currently being investigated in Phase 1/2 and 2 trials in patients with advanced solid tumor types that are associated with EphA2 expression, including metastatic pancreatic ductal adenocarcinoma (NCT04180371, NCT07450859)

## PLEASE VISIT MORE PRESENTATIONS ABOUT EPHA2-TARGETED BDCs AT AACR

- #LB012 Poster April 19
- #1325 Oral April 19
- #CT063 Poster April 20

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## ACKNOWLEDGMENTS

Medical writing support was provided by Tamara Fink, PhD, of Avalere Health Global Limited, which was funded by BicycleTx Ltd. Funding: This study was sponsored by BicycleTx Ltd. Presenting author: Lukas Stanczuk, lukas.stanczuk@bicyclerx.com. Presenting author COI disclosures: Lukas Stanczuk is an employee of Bicycle Tx and owns stocks/shares in the parent company, Bicycle Therapeutics, Inc.

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