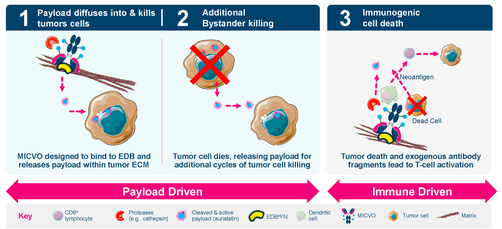
Evaluation of PYX-201, an EDB+FN-targeting ADC, in a comprehensive PDX mini-trial study enables identification of gene signatures associated with anti-tumor activity

Amanda Facklam, Justin Trickett, Sara Lewandowski, Chuan Shen, Frank Wang, Marsha Crochiere, Nicolas Severe, Jan Pinkas
Pyxis Oncology, Boston, Massachusetts, USA

Abstract: 3120

Background

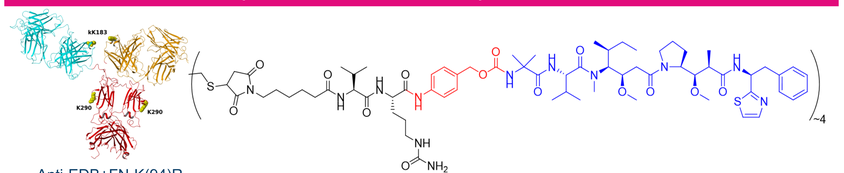
- PYX-201 (Micvotabart Pelidotin aka "MICVO"), a first-in-concept non-cellular targeting antibody-drug conjugate (ADC), is designed to bind specifically to extradomain-B of fibronectin (EDB+FN), a component of the extracellular matrix which is upregulated in various solid tumors with minimal to absent expression in normal adult tissues [1].
- PYX-201 is designed with a protease-cleavable valine-citrulline peptide linker, site-specific conjugation chemistry, and an optimized cytotoxic payload, Auristatin0101, resulting in improved stability and potency [1,2]. Extracellular proteases can cleave the valine-citrulline linker to release the payload into the tumor microenvironment [3].



Multiple mechanisms of action (MOA) may contribute to PYX-201 anti-tumor activity including direct tumor cell killing by released cytotoxic payload, bystander killing, and immunogenic cell death.

- **Objective: A comprehensive mini-trial study was designed to evaluate PYX-201 anti-tumor efficacy in patient-derived xenograft (PDX) models across ten solid tumor indications. Satellite tumors were collected from all models to determine which tumor properties at baseline may relate to PYX-201 activity.**
- PYX-201 (Micvotabart Pelidotin) is an investigational drug in a Phase 1 monotherapy trial (**NCT05720117**) and a Phase 1/2 combinational trial with pembrolizumab (**NCT06795412**) for advanced solid tumors.

PYX-201 (Micvotabart Pelidotin) Chemical Structure



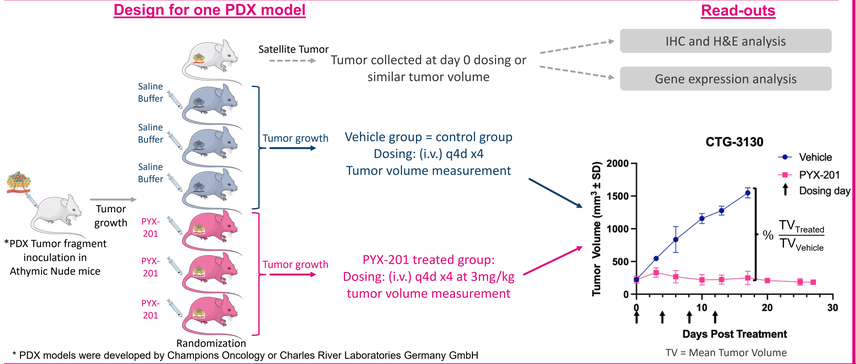
Anti-EDB+FN-K(94)R-huIgG1-K290C-κK183C | mc-Valine-citrulline linker | PABC Spacer | Auristatin0101 payload

PYX-201 MOA: Target Expression + Protease activity + Payload sensitivity

PYX-201 is a site-specific ADC with a drug antibody ratio of 4 (DAR = 4).
PYX-201 is composed of an anti-EDB+FN monoclonal antibody mAb (fully human IgG1) derived from the L19 clone. The antibody was engineered with cysteines κK183C and K290C for site-specific conjugation. The final mAb is defined as an anti-EDB+FN-K(94)R-huIgG1-K290C-κK183C. The Auristatin0101 payload was conjugated to the mAb via a mcValCitPABC linker [1].

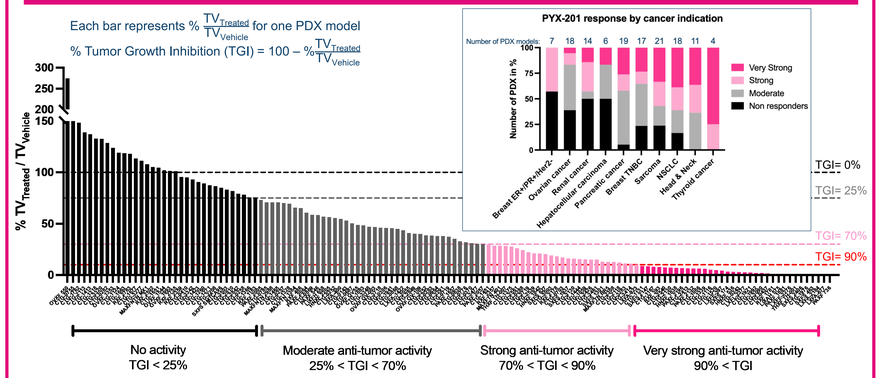
PDX Mini-Trial Design

Design for one PDX model | Read-outs



* PDX models were developed by Champions Oncology or Charles River Laboratories Germany GmbH

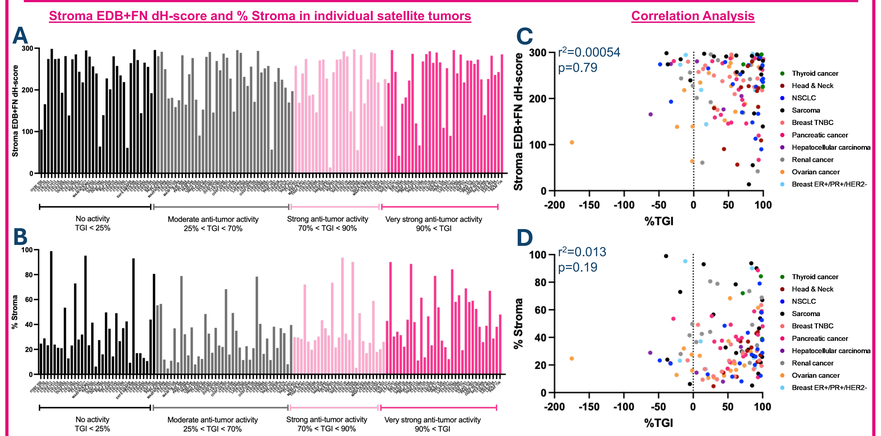
Broad PYX-201 anti-tumor activity observed across PDX Models

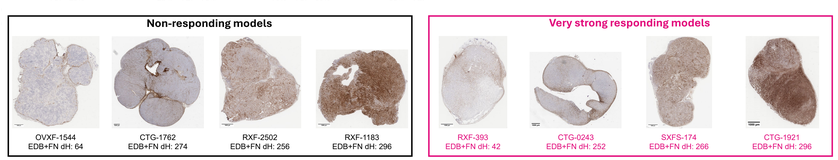


PYX-201 shows broad anti-tumor activity across ten solid tumor indications in PDX models.
The completed PDX mini-trial study includes 135 PDX models, updated from previous analysis of the first 108 models [4]. $\%TV_{Treated} / TV_{Vehicle}$ was calculated at the study endpoint (at least 2 mice remaining per group) for each PDX model. 45% of models have strong (70%<TGI<90%) to very strong (TGI>90%) activity, with only 25% of models showing no response (TGI<25%) to PYX-201. PDX models with very strong activity (TGI>90%) were found across 9/10 solid tumor indications.

EDB+FN is robustly expressed across PDX Models

Stroma EDB+FN dH-score and % Stroma in individual satellite tumors | Correlation Analysis





EDB+FN protein expression was assessed using a novel immunohistochemistry (IHC) assay.
A novel IHC assay and digital pathology algorithm were developed to quantify the intensity and distribution of EDB+FN protein expression in the tumor stroma, reported as stroma EDB+FN dH-scores [5]. Baseline satellite tumor samples from all PDX models in the Mini-Trial study were evaluated for EDB+FN protein expression (A) and stromal density (B) using this assay (models shown in order of increasing TGI).
EDB+FN is necessary but not sufficient for PYX-201 activity in PDX models.
These results indicate robust expression of EDB+FN across PDX models at baseline. However, no distinct correlation is observed between either EDB+FN protein expression (C) or stromal density (D) and PYX-201 anti-tumor activity, suggesting EDB+FN is necessary but not sufficient for PYX-201 anti-tumor efficacy in PDX models. Representative images of PDX tumors with low and high EDB+FN expression shown in E.

Enzyme and tumor stroma gene signatures are associated with PYX-201 activity in PDX models

Gene expression analysis reveals differential expression between PYX-201 response categories.
To further explore PYX-201 mechanisms of action, gene expression analysis was performed on RNA extracted from baseline satellite tumor samples from the PDX mini-trial study. Analysis was performed using the Nanostring ADC Development Panel. Differential expression analysis was performed comparing PDX models with very strong (TGI>90%) PYX-201 responses and PYX-201 non-responders (TGI<25%). Out of 750 total genes measured, 41 genes were differentially expressed. Gene set analysis was performed using Nanostring-defined gene categories to determine which sets may be associated with PYX-201 response in PDX samples. Gene sets with the greatest number of differentially expressed genes were tumor stroma and enzymes. Interestingly, upregulation of certain proteases may contribute to PYX-201 linker cleavage and therefore increased PYX-201 activity. Additionally, certain drug efflux pump genes were downregulated in very strong PDX responders.

Very Strong Responders vs. Non-Responders

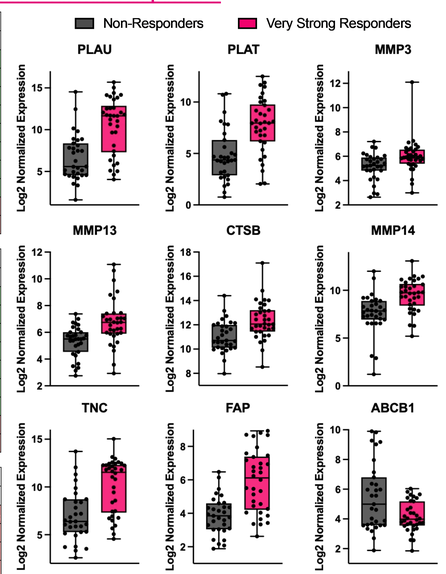
Enzymes		
Gene Name	Log2Fold Change	Protein Name
PLAU	4.72	Urokinase-type plasminogen activator
PLAT	4.19	Tissue-type plasminogen activator
MMP3	2.85	Matrix metalloproteinase-3
MMP13	1.76	Matrix metalloproteinase-13
CTSB	1.74	Cathepsin B
MMP14	1.51	Matrix metalloproteinase-14
CTSC	1.43	Cathepsin C
ADAM17	0.67	A disintegrin and metalloprotease 17
ADAM22	-2.23	A disintegrin and metalloprotease 22
MMP12	-4.72	Matrix metalloproteinase-12

Tumor Stroma		
Gene Name	Log2Fold Change	Protein Name
TNC	3.58	Tenascin C
FAP	3.20	Fibroblast activation protein alpha
MMP3	2.85	Matrix metalloproteinase-3
TIMP2	1.76	Metalloproteinase inhibitor 2
MMP13	1.76	Matrix metalloproteinase-13
MMP14	1.51	Matrix metalloproteinase-14
ADAM17	0.67	A disintegrin and metalloprotease 17
ADAM22	-2.23	A disintegrin and metalloprotease 22
MMP12	-4.72	Matrix metalloproteinase-12

Drug Efflux Pumps		
Gene Name	Log2Fold Change	Protein Name
ABCA3	-1.14	Phospholipid-transporting ATPase ABCA3
ABCA5	-2.15	Cholesterol transporter ABCA5
ABCC2	-2.56	Multidrug resistance-associated protein 2
ABCB1	-2.89	Multidrug resistance protein 1

Cutoffs: |log2 Fold Change|≥0.585, p-Adj≤ 0.05

Multidrug resistance protein 1 has been shown to cause drug resistance to monomethyl auristatin E payloads [6].



Conclusions

- **PYX-201 (Micvotabart Pelidotin) demonstrates broad anti-tumor activity in PDX models using immunodeficient mice, indicating strong activity of the Auristatin0101 payload across indications.**
- **In PDX models, robust protein expression of EDB+FN was observed by IHC. These data suggest EDB+FN is necessary but not sufficient for PYX-201 efficacy in PDX models and that other factors may contribute to PYX-201 activity.**
- **In PDX models, gene signatures for enzymes and tumor stroma in baseline tumors are associated with PYX-201 response. Furthermore, certain drug efflux pumps are downregulated in PDX models with very strong responses to PYX-201.**
- **Overall, multiple factors may contribute to PYX-201 activity including EDB+FN target expression, proteolytic activity for PYX-201 linker cleavage, and tumor responsiveness to the cytotoxic Auristatin0101 payload.**
- **Further multi-component analyses of factors including stroma, EDB+FN expression, and proteases and their relationship to PYX-201 efficacy in PDX models are ongoing.**

References

[1] Hooper et al., Mol Cancer Ther 2022 Sep 6;21(9):1462-1472.
[2] Graziani et al., Mol Cancer Ther 2020 Oct; 19(10):2068-2078.
[3] Lam et al., Cancer Res 2014 Oct; 74(19_Supplement):4837.
Servier Medical Art for figure design: https://smart.servier.com/
[4] Severe et al., Cancer Res 2024 March; 84(6_Supplement):742.
[5] Lewandowski et al., Cancer Res 2024 March; 84(6_Supplement):2908.
[6] Yu et al., Clin Cancer Res 2015 July; 21(14):3298-3306