Anti-tumor activity of micvotabart pelidotin is associated with enzyme gene expression in patient-derived xenograft sarcoma models

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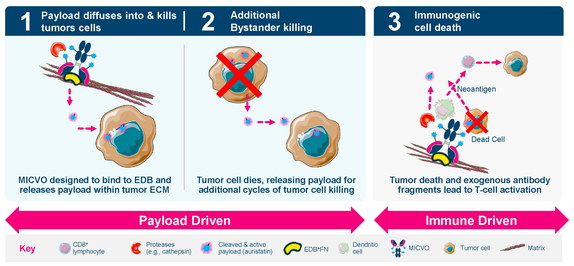
Abstract: 2165379

Background

- Micvotabart pelidotin (MICVO, aka PYX-201), a first-in-concept antibody drug conjugate (ADC), is designed to specifically target extradomain-B of fibronectin (EDB+FN) in the tumor extracellular matrix. EDB+FN is highly expressed in various solid tumors including sarcomas compared to normal adult tissues [1,2].
- MICVO is designed with a valine-citrulline linker, site-specific conjugation chemistry and an optimized cytotoxic payload, Auristatin0101 [1,3]. Extracellular proteases in the tumor microenvironment can cleave the linker to release the payload and kill tumor cells [4].



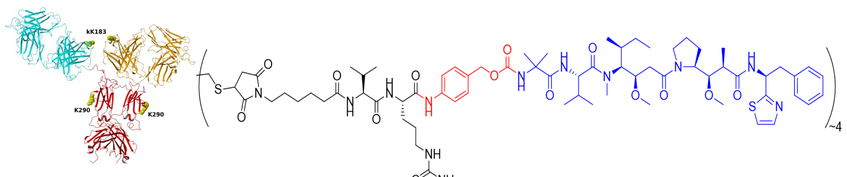
Multiple mechanisms of action (MOA) contribute to MICVO anti-tumor activity including direct tumor cell killing by released cytotoxic payload, bystander killing, and immunogenic cell death.

- Preliminary results from a Phase 1, Part 1 clinical trial (**NCT05720117**) demonstrated that MICVO exhibited favorable anti-tumor activity across multiple solid tumor types including sarcoma [5,6].
- Mesenchymal derivation and tumor heterogeneity of sarcomas hinder development of effective therapies. Interestingly, previous work demonstrated that EDB+FN is upregulated across a range of subtypes of sarcoma and that MICVO had strong anti-tumor activity in patient-derived xenograft (PDX) models representing a variety of sarcoma subtypes [7].
- **Objective: Building upon previous work demonstrating broad MICVO anti-tumor efficacy across PDX models representing various sarcoma subtypes, baseline tumors from these models were analyzed to elucidate which tumor properties at baseline may correlate to MICVO responsiveness.**

Methods

- 21 PDX sarcoma models were selected to test anti-tumor activity of MICVO across several sarcoma subtypes. The PDX models were developed by Champions Oncology or Charles River Laboratories Germany GmbH. Sarcoma PDX models having EDB+FN protein detected by immunohistochemistry (IHC) were selected. Mice were dosed with either vehicle or MICVO i.v. q4dx4 at 3mg/kg and tumor volume and body weights were measured. Two of the PDX sarcoma models were selected for a repeat study to evaluate long-term efficacy.
- For all 21 PDX models studied, a satellite tumor was collected from one mouse at randomization size. This tumor was analyzed with IHC staining to measure EDB+FN protein expression and with Nanostring RNA analysis to measure gene expression of a pre-defined panel of 750 genes (Nanostring ADC Development Panel).

MICVO chemical structure

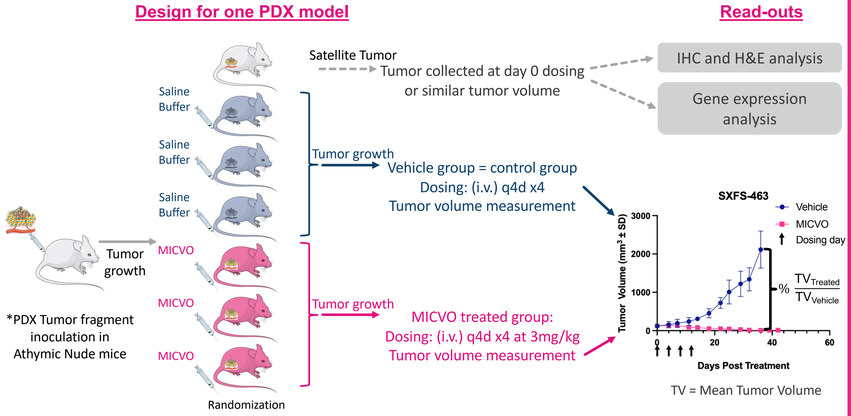


Anti-EDB+FN L19 κK183C+K94R+K290C | mc-Valine-citrulline linker | PABC Spacer | Auristatin0101 payload

MICVO MOA: Target Expression + Protease activity + Payload sensitivity

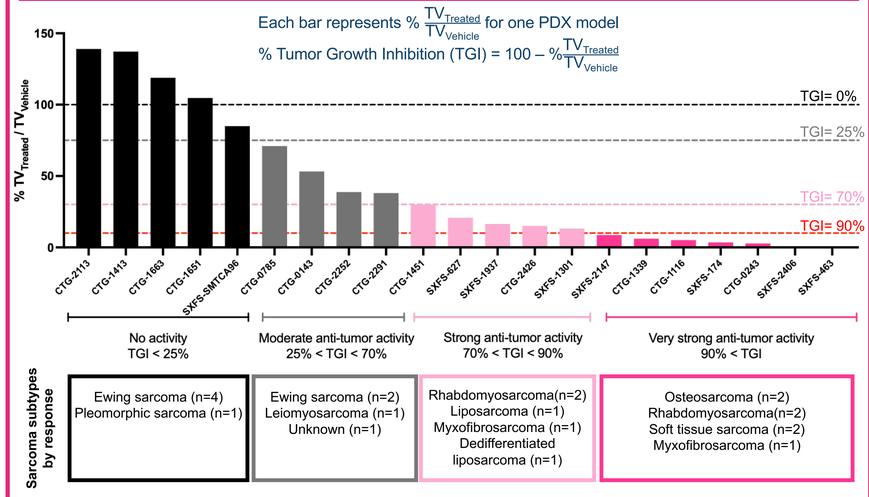
MICVO is a site-specific ADC with a drug antibody ratio of 4 (DAR = 4).
MICVO is composed of an anti-EDB+FN monoclonal antibody mAb (fully human IgG1) derived from the L19 clone. The antibody was engineered with cysteines κK183C and K290C for site-specific conjugation. The final mAb is defined as an anti-EDB+FN-K(94)R-huIgG1-K290C-κK183C. The Auristatin-0101 payload was conjugated to the mAb via a mcValCitPABC linker [1].

PDX mini-Trial design



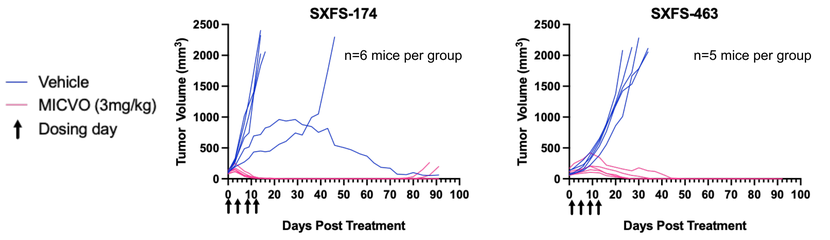
* PDX models were developed by Champions Oncology or Charles River Laboratories Germany GmbH

Broad MICVO anti-tumor activity across sarcoma PDX models



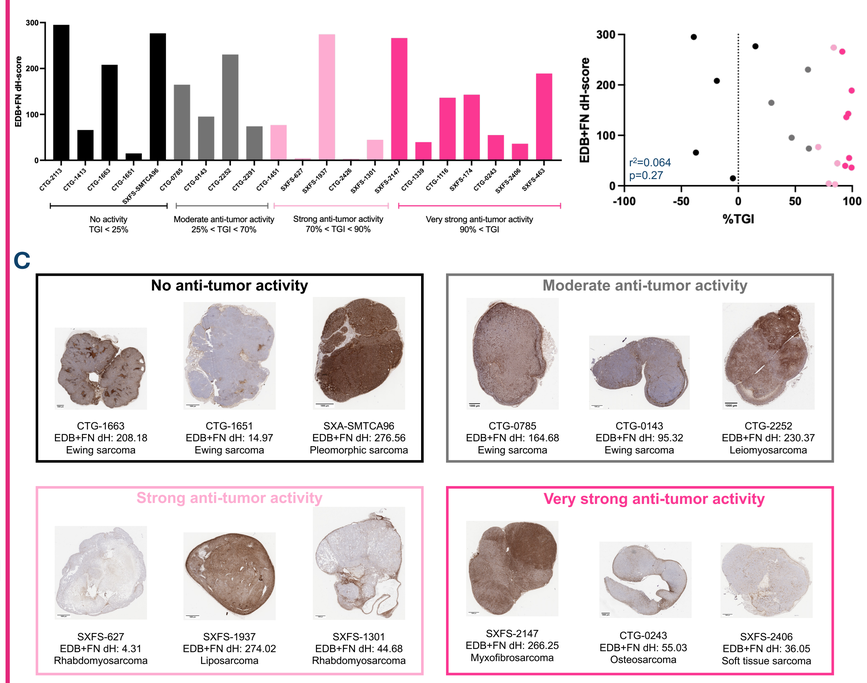
MICVO shows broad anti-tumor activity across sarcoma subtypes in PDX models.
$\%TV_{Treated} / TV_{Vehicle}$ was calculated at the study endpoint (at least 2 mice remaining per group) for each of the 21 sarcoma PDX models. % TGI enabled classification of PDX models into 4 response buckets: No activity (TGI<25%), moderate activity (25%<TGI<70%), strong activity (70%<TGI<90%), and very strong activity (TGI>90%). 57% (12/21) of models have strong or very strong activity (TGI>70%) including models across 6 sarcoma subtypes.

MICVO induces long-term anti-tumor efficacy



MICVO demonstrates long-term anti-tumor activity in sarcoma PDX models.
Two sarcoma PDX models which showed very strong activity were repeated for long-term evaluation. PDXs were monitored for 90 days with only 2/11 mice having tumor relapse after day 70. Spontaneous tumor regression was observed in one vehicle treated mouse.

Level of EDB+FN expression does not correlate with MICVO efficacy



EDB+FN is broadly expressed across baseline PDX sarcoma models and does not correlate with sensitivity to MICVO.
A novel IHC assay and digital pathology algorithm were developed to quantify the intensity and distribution of EDB+FN protein expression in the total tissue, reported as EDB+FN dH-scores [2]. A) Baseline satellite tumor samples from sarcoma PDX models were evaluated for EDB+FN protein expression using this assay (models shown in order of increasing TGI). These results indicate a wide range of EDB+FN expression across sarcoma subtypes at baseline. B) No distinct correlation is observed between EDB+FN protein expression and MICVO anti-tumor activity. C) Representative images of sarcoma PDX tumors with a range of EDB+FN expression.

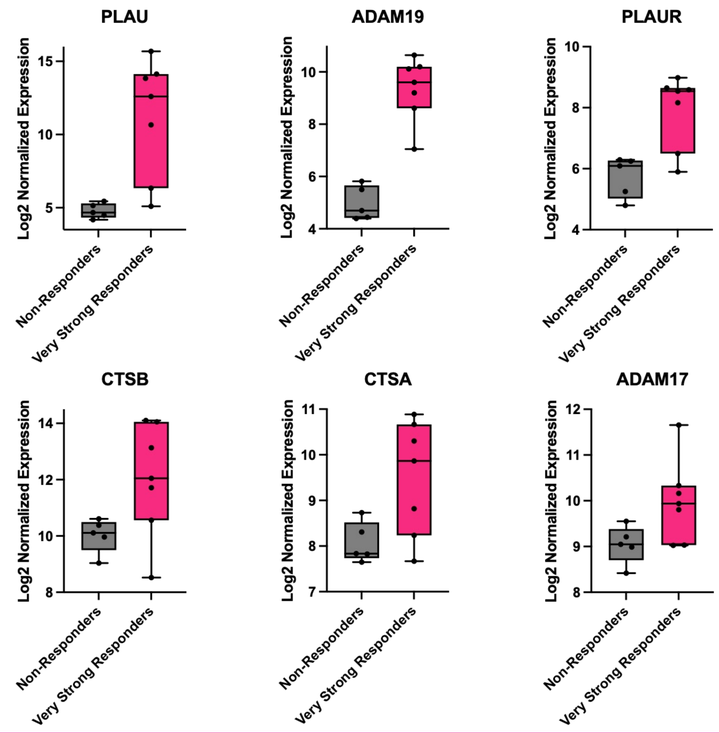
MICVO activity is associated with an enzyme gene signature

Gene expression analysis reveals differential expression between MICVO response categories.
To further explore MICVO mechanisms of action, gene expression analysis was performed on RNA extracted from baseline satellite tumor samples. Analysis was performed using the Nanostring ADC Development Panel. Differential expression analysis was performed comparing sarcoma PDX models with very strong (TGI>90%) MICVO responses and MICVO non-responders (TGI<25%). Out of 750 total genes measured, 25 genes were differentially expressed.
The enzyme gene signature is upregulated in very strong responders to MICVO.
Gene set analysis was performed using Nanostring-defined gene categories to determine which sets may be associated with MICVO response in sarcoma PDX samples. This analysis revealed that an enzyme gene signature is associated with response to MICVO. Notably, this enzyme signature contains proteases which may contribute to MICVO linker cleavage [8] and therefore lead to increased MICVO activity.

Very Strong Responders vs. Non-Responders

Enzymes		
Gene Name	**Log2Fold Change**	**Protein Name**
PLAU	9.37	Urokinase-type plasminogen activator
ADAM19	6.87	A disintegrin and metalloprotease 19
PLAUR	3.02	Urokinase-type plasminogen activator receptor
CTSB	2.81	Cathepsin B
CTSA	1.86	Cathepsin A
ADAM17	1.21	A disintegrin and metalloprotease 17

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



Conclusions

- **MICVO demonstrates broad anti-tumor activity across sarcoma subtypes in PDX models using immunodeficient mice, indicating strong activity of the Auristatin0101 payload across sarcoma subtypes.**
- **In sarcoma PDX models, robust EDB+FN protein expression was observed by IHC but had no clear association with MICVO response. These data suggest that additional factors may contribute to MICVO activity.**
- **In sarcoma PDX models, an enzyme gene signature in baseline tumors is associated with MICVO response. In alignment with MICVO MOA, expression of certain proteases, which may contribute to linker cleavage, were upregulated in very strong responders.**
- **Overall, multiple factors may contribute to MICVO activity including EDB+FN target expression, proteolytic activity for extracellular linker cleavage, and tumor responsiveness to the cytotoxic Auristatin0101 payload.**
- **Further integrated analyses of factors including EDB+FN expression and proteases and their relationship to MICVO efficacy in PDX models are ongoing.**

References

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[8] Shen et al., Mol Cancer Ther 2025 Oct; 24(10_Supplement):A116.
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