Characterization of micvotabart pelidotin target binding properties and extracellular payload release

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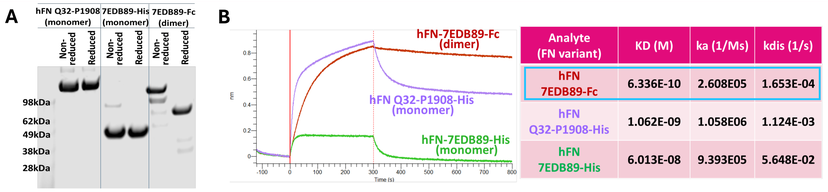
Abstract: A116

Background

- Extradomain-B of fibronectin (EDB+FN) is a non-cellular structural component within the tumor extracellular matrix (ECM) that is highly expressed in tumors compared to normal adult tissues [1, 2]. EDB+FN, a splice variant of fibronectin, is known to be involved in tumor angiogenesis, proliferation, and metastasis.
- Micvotabart pelidotin (MICVO, aka PYX-201) is a first-in-concept antibody-drug conjugate (ADC) targeting EDB+FN. MICVO is composed of a fully human IgG1 monoclonal antibody (mAb) with site-specific conjugation to an optimized Auristatin-0101 payload via a cleavable linker (DAR of 4) [1].
- MICVO binds to EDB+FN in the tumor ECM where extracellular proteases can cleave the valine-citrulline linker and release the cytotoxic payload to diffuse into and kill tumor cells. Preclinical studies demonstrated the broad antitumor activity of MICVO in solid tumor mouse models [1,3,4,5]. In addition, these studies showed that EDB+FN protein expression level did not correlate with MICVO efficacy in patient derived xenograft (PDX) models [5].
- Protease-mediated cytotoxic payload release into the tumor microenvironment resulting in tumor cell killing independent of ADC internalization and antigen density has been reported [6]. Recent data showed that an enzyme gene signature, including multiple cathepsins, was elevated in PDX models with very strong response to MICVO compared to PDX models which did not respond [5].
- **The objective of this poster is to further characterize the binding properties of MICVO and investigate the proteases that can cleave MICVO's linker to release its payload.**

[1] Hooper A et al., Mol Cancer Ther 2022 Sep 6;21(9):1462-1472.
[2] Lewandowski S et al., Cancer Res (2024) 84 (6_Supplement): 2908
[3] Severe N et al., Cancer Res (2024) 84 (6_Supplement): 742.
[4] Rodriguez A et al., Cancer Res (2025) 85 (8_Supplement_1): 3137.
[5] Facklam A et al., Cancer Res (2025) 85 (8_Supplement_1): 3120.
[6] Tsao L et al., Nat Commun 2025 Apr 2;16(1):3167.

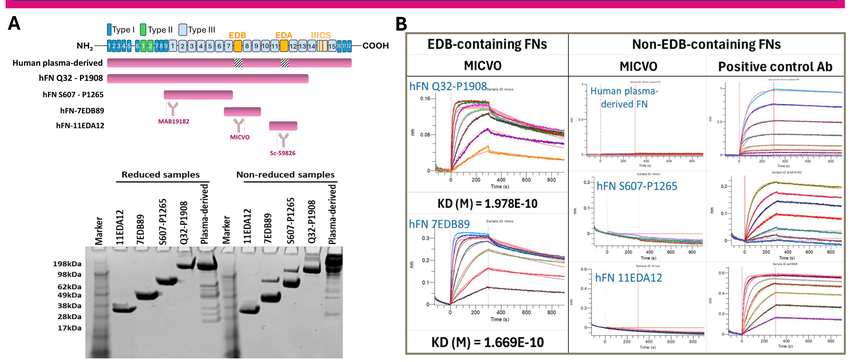
MICVO binding to EDB+FN is avidity driven



Analyte (FN variant)	KD (M)	ka (1/Ms)	kdis (1/s)
hFN 7EDB89-Fc	6.336E-10	2.608E05	1.653E-04
hFN Q32-P1908-His	1.062E-09	1.058E06	1.124E-03
hFN 7EDB89-His	6.013E-08	9.393E05	5.648E-02

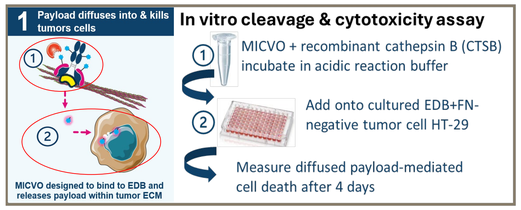
Stronger MICVO binding to dimeric EDB+FN variant than corresponding monomeric EDB+FN variant is attributed to the reduced dissociation rate. (A) SDS-PAGE gel analysis validated that recombinant monomeric EDB+FN protein (His-tagged hFN Q32-P1908 or 7EDB89) has the same molecular weight under non-reduced and reduced conditions. In contrast, recombinant dimeric EDB+FN protein (7EDB89-Fc fusion) has higher molecular weight under non-reduced condition compared to reduced condition. **(B)** Octet bio-layer interferometry (BLI) sensorgrams and the kinetic parameters for MICVO binding to monomeric and dimeric EDB+FN variants. 50 nM MICVO was captured onto AHC biosensors, and 50 nM recombinant EDB+FN variants were used as analytes. Dimeric 7EDB89-Fc molecule showed ~100-fold lower equilibrium dissociation constant (KD) than monomeric 7EDB89-His molecule, indicating a significantly enhanced interaction. The strong binding between MICVO and dimeric 7EDB89-Fc largely resulted from the slower dissociation rate (kdis) compared to both monomeric EDB+FN molecules, demonstrating the avidity driven binding of MICVO to its target.

MICVO specifically binds to EDB+FN



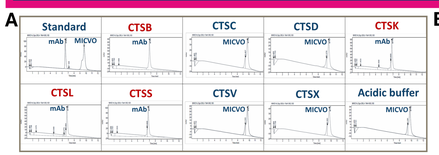
MICVO specifically binds to EDB-containing FNs, not to non-EDB-containing FNs. (A) Illustration of multiple FN variants with or without the EDB domain compared to the full-length FN molecular structure, including the epitopes targeted by MICVO and positive control antibodies (Abs). SDS-PAGE gel confirmed the size of the FN variants using both reduced and non-reduced samples. **(B)** Octet BLI sensorgrams for anti-FN Abs binding with FN variants. 50 nM His-tagged recombinant FN variant proteins (hFN Q32-P1908, S607-P1265, 7EDB89 & 11EDA12) were captured onto NTA biosensors, and 0 ~ 25 nM MICVO & positive control Abs were used as analytes, respectively. For human plasma-derived FN, 0 ~ 25 nM MICVO & the positive control Ab MAB19182 were captured onto AHC & AMC biosensors, respectively, and 50 nM human plasma-derived FN was used as an analyte. These results showed that MICVO specifically bound to the EDB-containing FN variants and did not bind to non-EDB-containing FN variants, such as plasma FN and EDA+FN, demonstrating the high specificity of MICVO.

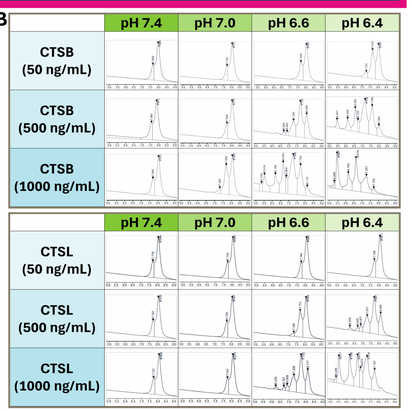
EDB+FN-negative cancer cells can be killed by in vitro extracellularly released MICVO payload



CTSB-mediated in vitro extracellular payload release enables the killing of EDB+FN-negative tumor cells. (A) Experimental design of in vitro payload release by recombinant CTSB and cytotoxicity assay using the EDB+FN-negative cell line HT-29, which mimics the non-cellular targeting mechanism of MICVO. **(B)** In HT-29 cytotoxicity assay, 1.6 µg/mL MICVO & 1 µg/mL recombinant CTSB were incubated in the activation buffer (pH5) for two hours. The mixture caused significant cell death after 4-days, whereas no cell killing was observed for all control groups shown in the graph, demonstrating that the killing of EDB+FN-negative HT-29 cells was specifically caused by CTSB-mediated extracellular payload release of MICVO.

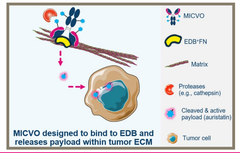
Various cathepsins can release MICVO payload in vitro





Cathepsin-mediated MICVO payload release is concentration and pH dependent. (A) Recombinant cathepsin (CTS) proteins (4 µg/mL) were mixed with MICVO (1 µg/mL) individually in 1xPBS buffer with vendor-provided pH for enzymatic activity. The mixtures were incubated under 37°C for 4 hours and then injected into hydrophobic interaction chromatography (HIC) column using Agilent 1260 Infinity II LC System. CTSB, CTSK, CTSL & CTSS (red font) showed efficient MICVO payload release. **(B)** Recombinant CTSB & CTSL proteins at different concentrations were incubated with 1µg/mL MICVO under a range of pH conditions (pH7.4 ~ 6.4) at 37°C overnight. A clear concentration and pH dependent release of MICVO payload was observed. No cleavage was observed under neutral pH7.4.

Proposed non-cellular activation of MICVO



- MICVO binds to EDB+FN in the ECM of the tumor.
- pH within the tumor is low (due to the insufficient oxygen supply) enabling the proteolytic activity of cathepsins.
- Extracellular cathepsins (and other potential proteases) cleave the linker-payload releasing the payload to diffuse into and kill tumor cells.

Conclusions

- MICVO binds to EDB+FN in an avidity driven manner, which supports the hypothesis of the stronger interaction with oligomeric fibril structure and longer residence time of MICVO in tumor ECM.
- MICVO binds to EDB+FN with high specificity, indicative of the desirable pharmacokinetics (PK) and minimal off-target effects observed clinically.
- The non-cellular targeting mechanism of MICVO in tumors is supported by 1) in vitro cleavage of the MICVO linker by several recombinant proteases in a concentration and pH dependent manner and 2) killing of cells that do not express the antigen for MICVO by extracellularly released payloads, consistent with recently published data on the extracellular cleavage of Enhertu [6].
- Additional in vitro and in vivo studies will be conducted to further investigate cathepsin-mediated extracellular MICVO payload release.
- These data support MICVO's utility as an investigational therapy for patients with difficult-to-treat cancers in the ongoing clinical trials as a monotherapy (NCT05720117) and in combination with pembrolizumab (NCT06795412).

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