Sarcomas express extra domain B of fibronectin and preclinical patient-derived xenograft models of several sarcoma subtypes are sensitive to the stroma targeting antibody drug conjugate PYX-201

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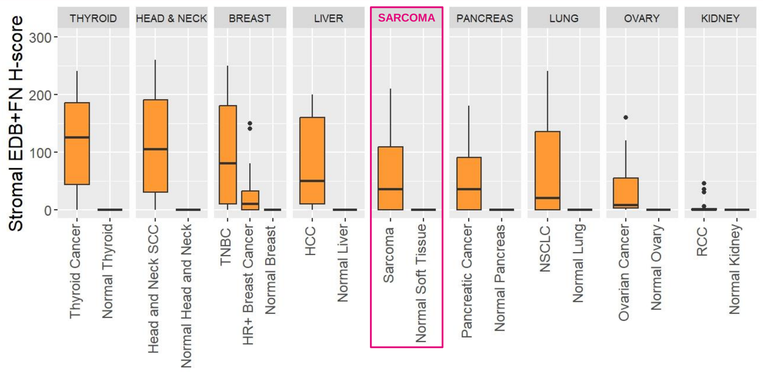
BACKGROUND & OBJECTIVE

- Extra domain B of fibronectin (EDB+FN), a splice-variant of fibronectin, is abundantly expressed in the extracellular matrix (ECM) of many solid tumors including sarcoma, with absent or low expression in normal adult tissue, making EDB+FN an intriguing target for drug delivery to several tumors including sarcoma [1] [2].
- PYX-201, a first-in-concept antibody-drug conjugate (ADC), is designed to target EDB+FN which is a noncellular antigen found in the ECM. Extracellular proteases cleave the linker to release the toxic payload Auristatin0101 into the tumor microenvironment which induces anti-tumor activity by diffusing into and killing tumor cells. These tumor cells then release payload to kill additional tumor cells via the bystander effect as well as elicit an immunogenic response resulting from release of neoantigens from apoptosed tumor cells.
- The potential for PYX-201 to demonstrate clinical benefit for patients with solid tumors, including sarcoma, is currently being evaluated in a global phase 1 clinical trial (NCT05720117).
- Sarcoma are mesenchymally-derived cancers that are usually rich in extracellular matrix proteins. The high tumor heterogeneity exhibited by sarcomas complicate treatment options which are often ineffective.
- The objective of this study was to expand the understanding of EDB+FN expression and prevalence across human sarcoma subtypes, as well as evaluate the anti-tumor activity of PYX-201 in patient-derived xenograft (PDX) pre-clinical mouse models across many different sarcoma subtypes.

METHODS

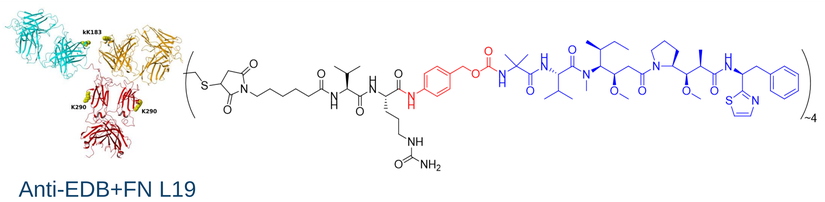
- To characterize the prevalence and distribution of EDB+FN expression in solid tumor types being explored in the Phase 1 PYX-201-101 clinical study and to evaluate clinical biopsies, an immunohistochemistry (IHC) assay for detection of a dynamic range of EDB$^+$FN protein expression in formalin-fixed paraffin-embedded (FFPE) tumor tissue and a modified H-score that incorporated intensity and distribution by a pathologist were previously developed [3].
- A digital pathology algorithm was also developed for streamlined assessment of EDB$^+$FN protein expression in samples from tumor tissue microarrays (TMAs). Sarcoma TMAs were commercially sourced for all subtypes except for chordoma, which was generously provided by the Chordoma Foundation. For TMA samples, digital H (dH)-scores, representing presence of EDB+FN, were calculated for the total evaluable tissue area of each sample.
- A PDX mini-trial was designed to test anti-tumor activity of PYX-201 in several sarcoma subtypes. The PDX models were developed by Champions Oncology or Charles River Laboratories Germany GmbH. Models were selected based on expression of EDB+FN by IHC. Mice were dosed with either vehicle or PYX-201 i.v. q4d x4 at 3mg/kg and tumor volume and body weights were measured.

EDB+FN is broadly expressed in the stroma of many solid tumor types



EDB$^+$FN is detected in tumor stroma by IHC with a broad range of expression within and across many solid tumor indications, including sarcoma, with little to no expression in normal tissues.

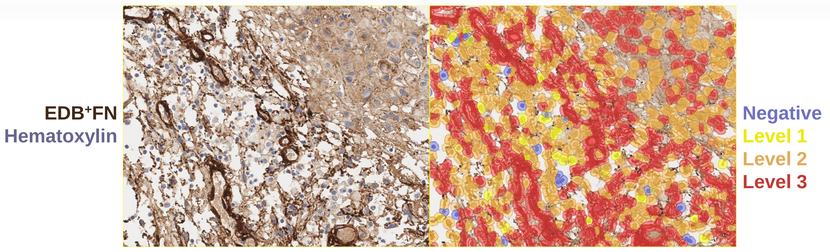
PYX-201 CHEMICAL STRUCTURE



Anti-EDB+FN L19 K94R+κK183C+K290C — Linker — Spacer — Payload

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 Auristatin-0101 payload was conjugated to the mAb via a mcValCitPABC linker [2].

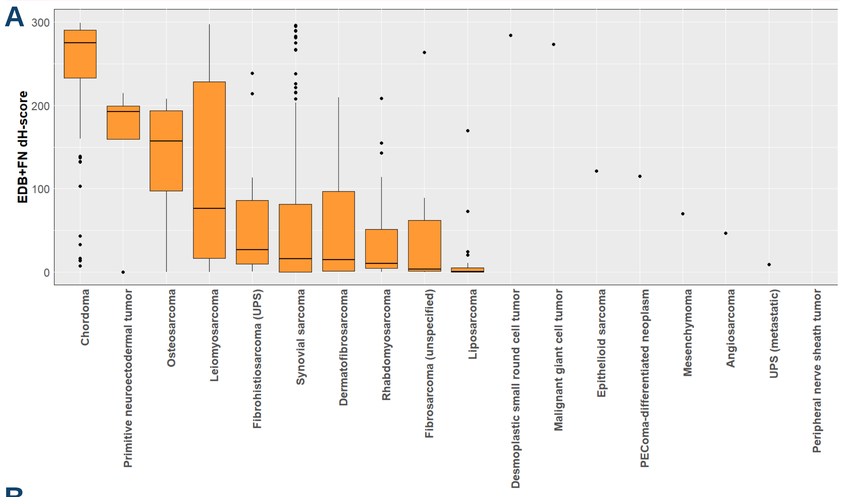
Detection of EDB+FN protein in sarcoma subtypes using digital pathology



Development of a digital pathology algorithm for scoring EDB$^+$FN IHC for TMAs. Thresholding was applied with intensity cutoffs for each EDB$^+$FN expression level set using pathologist scores as guides. Combined areas of cells in each intensity threshold were used to compute EDB$^+$FN dH-scores. Image shown is an example from the Chordoma Foundation chordoma TMA.

EDB+FN is broadly expressed across many sarcoma subtypes

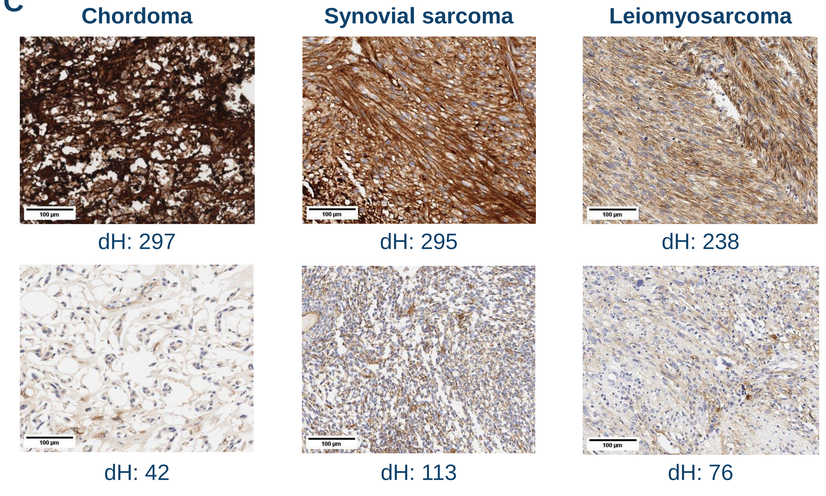
A



B

Indication	N	Median dH	Range
Chordoma	94	275	7-299
Primitive neuroectodermal tumor	5	192	0-215
Osteosarcoma	5	157	0-208
Leiomyosarcoma	29	76	0-297
Fibrohistiosarcoma (UPS)	12	27	1-238
Synovial sarcoma	134	16	0-296
Dermatofibrosarcoma	10	15	1-209
Rhabdomyosarcoma	23	10	0-208
Fibrosarcoma (unspecified)	9	4	0-263
Liposarcoma	27	1	0-170
Peripheral nerve sheath tumor	2	-	-
UPS (metastatic)	1	-	-
Angiosarcoma	1	-	-
Desmoplastic small round cell tumor	1	-	-
Epithelioid sarcoma	1	-	-
Malignant giant cell tumor	1	-	-
Mesenchymoma	1	-	-
PEComa-differentiated neoplasm	1	-	-

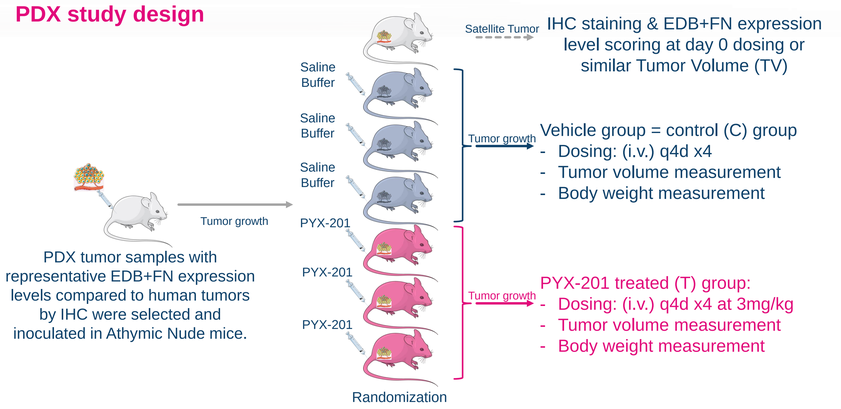
C



Broad range of EDB+FN expression is observed across sarcoma subtypes. A. EDB+FN protein expression is represented by dH score and was observed in most sarcoma subtypes. Evaluation was limited to those subtypes that were available commercially, many of which had limited numbers of samples, except for chordoma which was generously provided by the Chordoma Foundation and included samples from 94 patient donors. Chordoma subtype showed the most consistently high expression of EDB+FN. B. Table of the sarcoma subtypes in A including the number of samples, median dH score and range for each subtype, where available. C. Representative images of EDB+FN staining in 20X regions of interest representing high (dH >200) and low (dH < 125) expressing chordoma, synovial sarcoma, and leiomyosarcoma tissues.

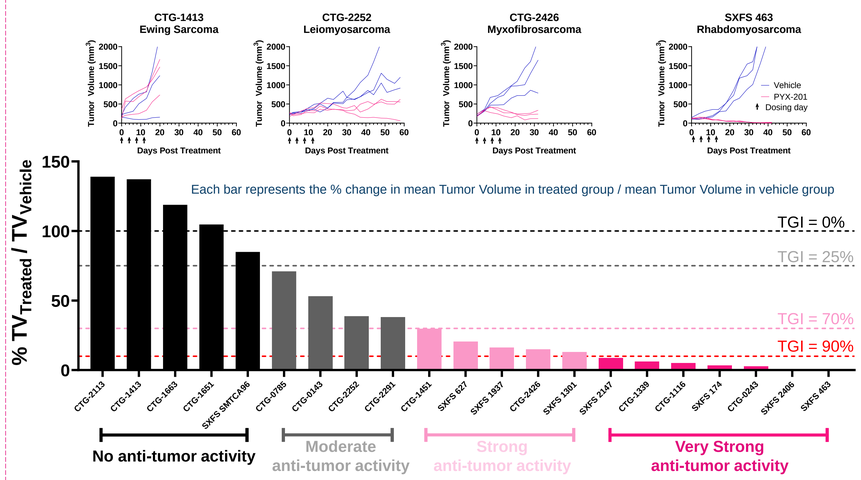
Broad PYX-201 anti-tumor activity across a panel of human sarcoma PDX models determined by calculation of tumor growth inhibition at study end

PDX study design



Calculate %T/C ratio ($TV_{treated}/TV_{control}$), Tumor Growth Inhibition (TGI=100-T/C), Tumor Regression (PD, SD, PR, CR) to evaluate PYX-201 anti-tumor activity

PYX-201 anti-tumor activity is observed in pre-clinical sarcoma PDX models across different tumor subtypes: % Tumor growth inhibition (TGI) was calculated from the % $TV_{treated}$ / $TV_{vehicle}$ ratio at the terminal endpoint for the vehicle group (at least n=2 mice/group). Comparison of tumor growth in PYX-201 Treated group vs. Vehicle group allowed each PDX model to be sorted into 4 groups: No anti-tumor activity (TGI<25%), Moderate anti-tumor activity (25%<TGI<70%), Strong anti-tumor activity (<70%TGI<90%) and Very Strong anti-tumor activity (90%<TGI). These data show that 57%* of sarcoma PDX models have strong to very strong PYX-201 anti-tumor activity. * 3 PDX models have been added to the study since the abstract submission



Tumor regression quantification in individual mice in the PYX-201 treated group: **Complete Response (CR):** Disappearance of measurable tumor for 2 or more consecutive measurements. **Partial Response (PR):** ≥ 30% decrease in tumor volume from largest tumor volume for 2 consecutive measurements. **Progressive Disease (PD):** ≥ 20% increase in tumor volume from baseline at study endpoint and no sufficient decrease during the study to qualify for PR.

PDX	Subtype	Regression	PDX	Subtype	Regression	PDX	Subtype	Regression	PDX	Subtype	Regression
CTG-2113	Ewing Sarcoma	PD: 2 PR: 1	CTG-0785	Ewing Sarcoma	PD: 3	CTG-1451	Dedifferentiated liposarcoma	PD: 2 PR: 1	SXFS 2147	Myxofibrosarcoma	PR: 2 CR: 1
CTG-1413	Ewing Sarcoma	PD: 3	CTG-0143	Ewing Sarcoma	PD: 3	SXFS 627	Rhabdo-myosarcoma	PD: 2 PR: 1	CTG-1339	Osteosarcoma	PR: 3
CTG-1663	Ewing Sarcoma	PD: 2 PR: 1	CTG-2252	Leiomyosarcoma	PD: 1 PR: 2	SXFS 1937	Liposarcoma	PD: 1 PR: 2	CTG-1116	Rhabdo-myosarcoma	PR: 2 CR: 1
CTG-1651	Ewing Sarcoma	PD: 3	CTG-2291	Unknown	PD: 1 PR: 2	CTG-2426	Myxofibrosarcoma	PR: 3	SXFS 174	Soft Tissue Sarcoma	PR: 2 CR: 1
SXFS SMTCA 96	Pleomorphic sarcoma	PD: 3				SXFS 1301	Rhabdo-myosarcoma	PR: 1 CR: 2	CTG-0243	Osteosarcoma	PR: 3
									SXFS 2406	Soft Tissue Sarcoma	PR: 1 CR: 2
									SXFS 463	Rhabdo-myosarcoma	PR: 2 CR: 1

CONCLUSIONS

- EDB+FN is upregulated in sarcoma with a wide range of expression intensity across the limited subtypes available for evaluation. EDB+FN is thus an intriguing target for drug delivery in many sarcoma tumor subtypes and further investigation into additional subtypes is warranted.
- PYX-201 is well tolerated in PDX models of sarcoma with strong anti-tumor activity seen across a variety of sarcoma subtypes.
- The potential for PYX-201 to demonstrate clinical benefit for patients with sarcoma and other solid tumors is currently being evaluated in a global phase 1 clinical trial (NCT05720117).

REFERENCES

[1] Hooper et al., Mol Cancer Ther 2022 Sep 6;21(9):1462-1472 [2] AACR 2024 Abstract #2908 [3] AACR 2024 Abstract #742

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