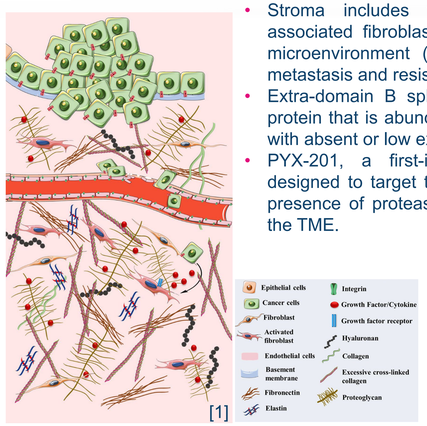
PYX-201, a stroma-targeting ADC composed of an anti-EDB+FN antibody conjugated to Auristatin0101, demonstrates strong anti-tumor efficacy across multiple human cancer indications in pre-clinical PDX tumor models

Nicolas Severe[1], Amanda Facklam[1], Liz McMichael[1], Biplab Das[1], Sara Lewandowski[1], Justin Trickett[1], Liyang Diao[1], Chengfeng Merriman[1], Chuan Chen[1], Jianwen Feng[1], Shawn Harriman[1], Marsha Crochiere[1], Philipp Steiner[1], Jan Pinkas[1]

[1]Pyxis Oncology, Boston, MA

Abstract #: 742

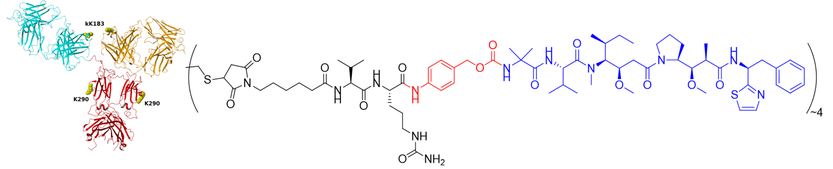
Background



[1]

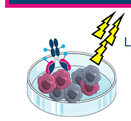
- Stroma includes the extracellular matrix, vasculature, cancer-associated fibroblasts and mesenchymal stromal cells in the tumor microenvironment (TME) and is crucial to support tumor growth, metastasis and resistance to treatment [1].
- Extra-domain B splice variant of fibronectin (EDB+FN) is a matrix protein that is abundantly expressed in the TME of many solid tumors with absent or low expression in normal adult tissue [2].
- PYX-201, a first-in-concept antibody-drug conjugate (ADC), is designed to target tumor stroma by binding to EDB+FN and then, in presence of proteases, releasing its toxic payload extracellularly into the TME.
- PYX-201 is designed to have improved plasma stability, better potency and tumor permeability due to optimized payload, linker technology and site-specific conjugation chemistry [2,3].
- Potential mechanisms of PYX-201 anti-tumor activity: 1) Toxic payload released in the TME diffuses into and kill tumor cells, 2) recycled payload released from dying tumor cell resulting in additional bystander activity and 3) immunogenic cell death.
- PYX-201 is an investigational drug currently in Phase I Clinical Trial (NCT05720117)

PYX-201 Chemical Structure

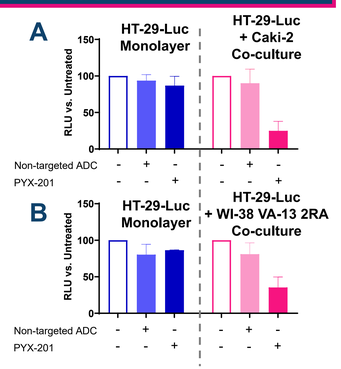


PYX-201 is a site-specific ADC with a drug antibody ratio of 4 (DAR = 4).
PYX-201 is composed of an anti-EDB+FN monoclonal antibody mAb (fully human IgG1) derived from the L19 clone. The antibody was engineered with cysteines κK183C and K290C for site-specific conjugation. The final mAb is defined as an anti-EDB+FN-K(94)R-huIgG1-K290C-κK183C. The Auristatin-0101 payload was conjugated to the mAb via a mcValCitPABC linker [2].

Bystander Activity of PYX-201 In-Vitro



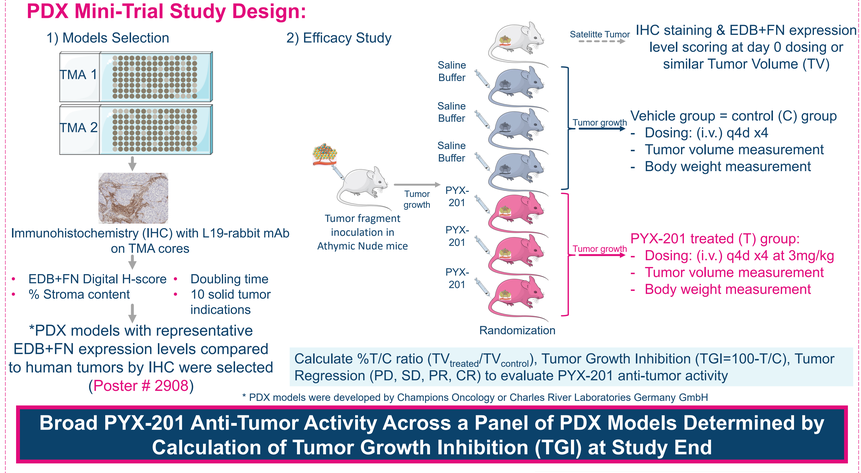
Proof-of-concept bystander activity of PYX-201 in a co-culture assay
HT-29 cells which are negative for EDB+FN were modified to express the Luciferase reporter and cultured as a monolayer or mixed with (**A**) Caki2 or (**B**) WI-38 VA-13 2RA (both are positive for EDB+FN) at a 1:3 ratio. Cells were treated with a non-targeted ADC or PYX-201 for 5 days at 8,000 ng/ml and Luciferase activity was measured to evaluate the amount of HT-29 cells in each well. HT-29 cells were sensitive to PYX-201 only when co-cultured with EDB+FN positive cells. The non-targeted ADC did not induce cell killing compared to the untreated condition.
These data demonstrate the bystander effect of PYX-201 in an in-vitro setting.



PDX Mini-Trial Study Objective and Design

Objective: Determine the breadth of PYX-201 anti-tumor activity across a panel of patient-derived xenograft (PDX) models

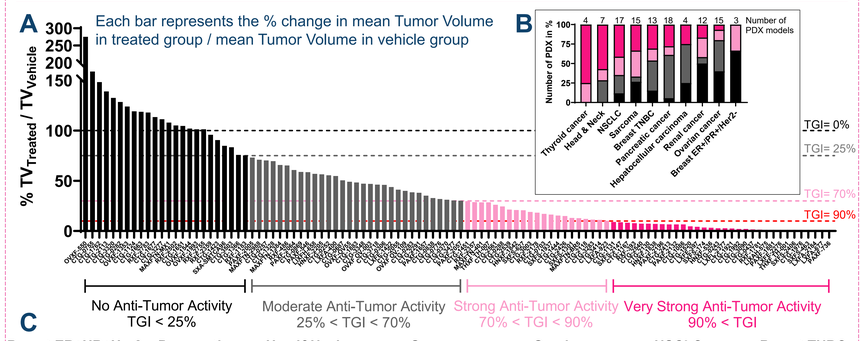
PDX Mini-Trial Study Design:

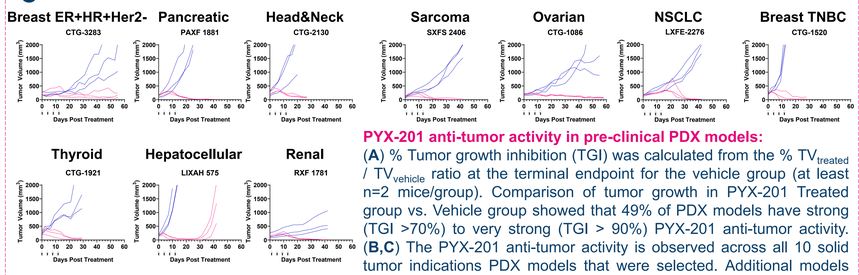


Calculate %T/C ratio ($TV_{treated}/TV_{control}$), Tumor Growth Inhibition (TGI=100-T/C), Tumor Regression (PD, SD, PR, CR) to evaluate PYX-201 anti-tumor activity

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

Broad PYX-201 Anti-Tumor Activity Across a Panel of PDX Models Determined by Calculation of Tumor Growth Inhibition (TGI) at Study End





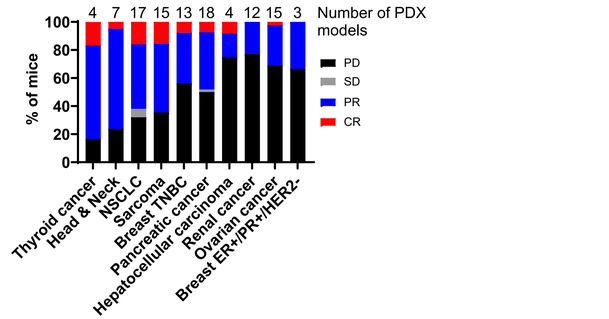
PYX-201 anti-tumor activity in pre-clinical PDX models:
(**A**) % Tumor growth inhibition (TGI) was calculated from the % $TV_{treated}$ / $TV_{vehicle}$ ratio at the terminal endpoint for the vehicle group (at least n=2 mice/group). Comparison of tumor growth in PYX-201 Treated group vs. Vehicle group showed that 49% of PDX models have strong (TGI >70%) to very strong (TGI > 90%) PYX-201 anti-tumor activity. (**B,C**) The PYX-201 anti-tumor activity is observed across all 10 solid tumor indications PDX models that were selected. Additional models are being tested.

These data demonstrate broad PYX-201 anti-tumor activity across multiple human cancer indications in pre-clinical PDX models.

PYX-201 induces tumor regression across a panel of PDX models

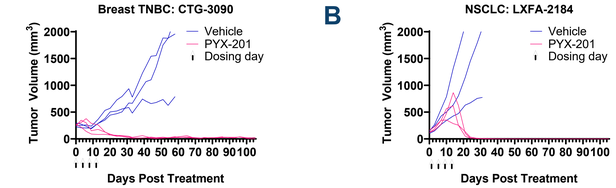
Tumor regression quantification in individual mice in the PYX-201 treated group:

- **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
- **Stable Disease (SD):** Neither sufficient decrease or increase in tumor size to qualify as a PR or PD



These data revealed the broad PYX-201 anti-tumor activity across multiple human cancer indications in pre-clinical PDX models.

PYX-201 induces long-term anti-tumor activity



(**A**) One Breast TNBC and (**B**) one NSCLC PDX models that showed very strong PYX-201 anti-tumor activity were randomly selected for long-term evaluation. PDXs were monitored for >100 days, and no tumor relapse was observed demonstrating the long-term anti-tumor activity of PYX-201.

Conclusions

- **PYX-201 can kill EDB+FN negative cancer cells in a co-culture assay, demonstrating PYX-201 bystander activity in-vitro.**
- **The PDX Mini-Trial study identified a variety of pre-clinical tumor indications with strong PYX-201 anti-tumor activity and tumor regression. Data will continue to evolve as additional PDX models are being tested.**
- **Analyses evaluating potential correlations between EDB+FN expression and/or stroma density with PYX-201 anti-tumor activity are on-going.**

References

[1] Jurj et al., J Exp Clin Cancer Res 2022 Sep 16;41(1): 276.
[2] Hooper et al., Mol Cancer Ther 2022 Sep 6;21(9):1462-1472.
[3] Graziani et al., Mol Cancer Ther 2020 Oct; 19(10):2068-2078.
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Contact Info: Presenting & Primary Author Nicolas Severe nsevere@pyxisoncology.com