The combination of anti-PD1 and a mouse analog of PYX-201, an antibody-drug conjugate targeting the extra-domain B splice variant of fibronectin (EDB+FN), shows greater anti-tumor efficacy than either treatment alone

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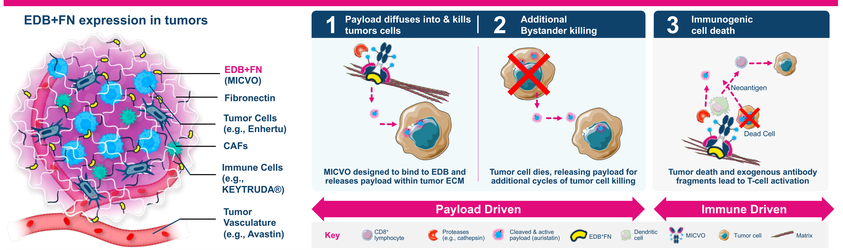
Abstract: 3137

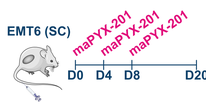
Background

- Antibody-drug conjugates (ADCs) are transforming cancer therapy, with some enhancing the efficacy of checkpoint immunotherapy (**1, 2**).
- The extra-domain B splice variant of fibronectin (EDB+FN) is minimally expressed in normal adult tissues but highly upregulated in tumors, making it a promising therapeutic target (**3, 4**).
- PYX-201 (Micvotabart Pelidotin, aka "MICVO") is a first-in-concept ADC targeting the non-cellular EDB+FN and is an investigational drug currently being evaluated in a Phase 1 monotherapy trial (**NCT05720117**) and a Phase 1/2 combination trial with pembrolizumab (**NCT06795412**) for advanced solid tumors.
- Preclinical studies have demonstrated that PYX-201 has broad anti-tumor activity across various patient-derived xenograft models (**5**).
- A mouse analog of PYX-201 (defined as maPYX-201), composed of the L19-derived variable regions fused to a mouse IgG2a backbone and conjugated to an optimized Auristatin0101 payload via an mcValCitPABC cleavable linker with an average drug-to-antibody ratio of 4, enhanced the sensitivity of a syngeneic breast tumor model to anti-PD-L1 immunotherapy (**4**).
- **The objective of this poster is to demonstrate that maPYX-201 modulates the tumor immune microenvironment, enhancing sensitivity to anti-PD1 in a syngeneic breast tumor model.**

Mechanism of Action for PYX-201 (Micvotabart Pelidotin)



Methods



- Eight-week-old female Balb/c mice were subcutaneously (SC) injected with 1.0 × 10^6 EMT6 breast cancer cells into the right flank.
- Once tumors reached ~150 mm^3 (Day 0), mice were randomized and treated with saline or maPYX-201 (Q4Dx3).
- Body weight and tumor volumes were monitored to assess drug tolerability and tumor growth inhibition, respectively.
- At select timepoints, tumors were harvested, enzymatically digested for flow cytometry, or fixed and paraffin-embedded for immunohistochemistry (IHC) or immunofluorescence (mIF).

maPYX-201 inhibits EMT6 tumor outgrowth with good tolerability

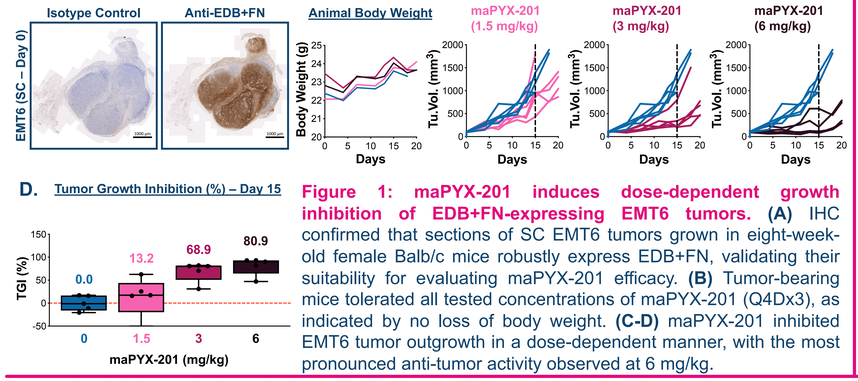
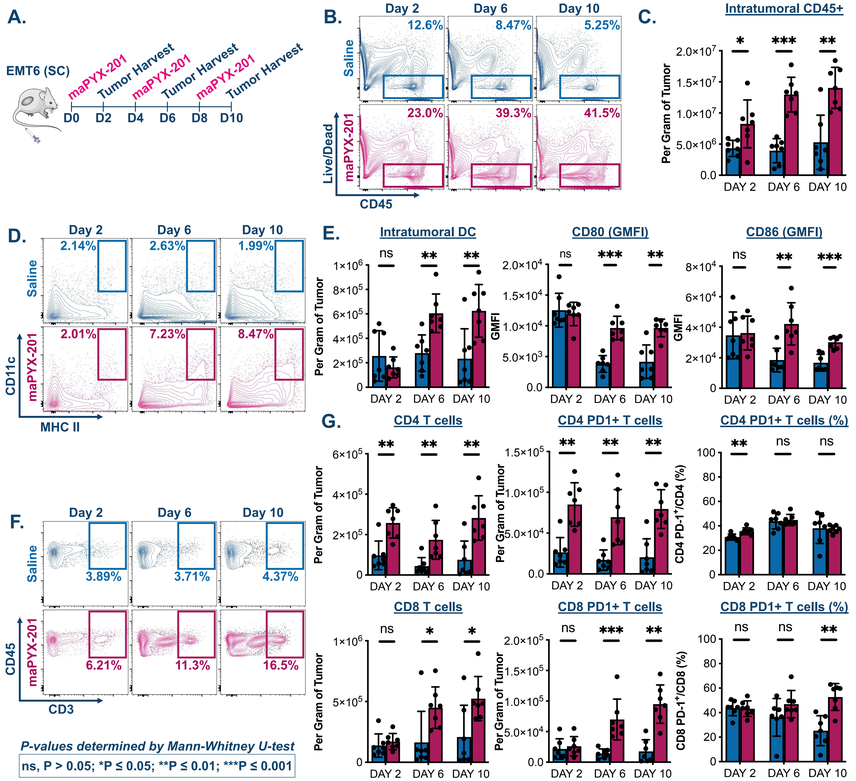


Figure 1: maPYX-201 induces dose-dependent growth inhibition of EDB+FN-expressing EMT6 tumors. (A) IHC confirmed that sections of SC EMT6 tumors grown in eight-week-old female Balb/c mice robustly express EDB+FN, validating their suitability for evaluating maPYX-201 efficacy. **(B)** Tumor-bearing mice tolerated all tested concentrations of maPYX-201 (Q4Dx3), as indicated by no loss of body weight. **(C-D)** maPYX-201 inhibited EMT6 tumor outgrowth in a dose-dependent manner, with the most pronounced anti-tumor activity observed at 6 mg/kg.

maPYX-201 enables infiltration of activated immune cells into tumors



P-values determined by Mann-Whitney U-test

ns, P > 0.05; *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001

Figure 2: maPYX-201 drives dendritic cell (DC) activation and T cell infiltration into tumors. (A) EMT6 tumors from saline-treated (blue) and 3 mg/kg maPYX-201-treated (pink) mice were harvested 48-hours post-treatment, weighed, and processed for flow cytometry. **(B-G)** Two days after the first treatment, maPYX-201 increased the infiltration of CD45+ immune cells into tumors, including DCs and PD1+ T cells, and this effect persisted 10 days post-treatment. Additionally, DCs exhibited increased geometric mean fluorescence intensity (GMFI) of co-stimulatory molecules, indicating activation.

maPYX-201 drives T cell infiltration and widespread distribution in tumors

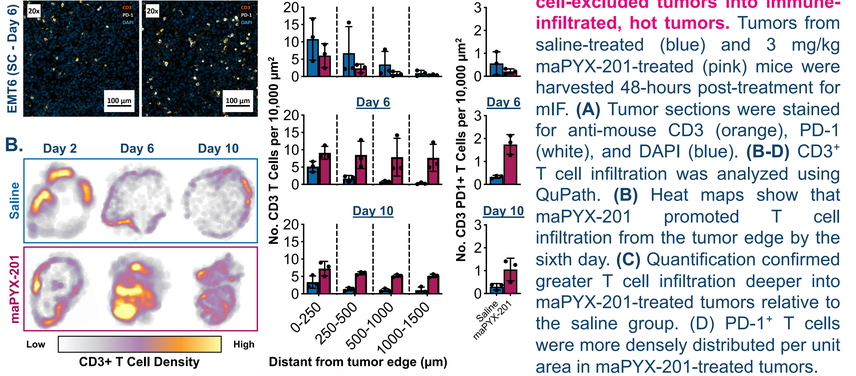


Figure 3: maPYX-201 converts T cell-excluded tumors into immune-infiltrated, hot tumors. Tumors from saline-treated (blue) and 3 mg/kg maPYX-201-treated (pink) mice were harvested 48-hours post-treatment for mIF. **(A)** Tumor sections were stained for anti-mouse CD3 (orange), PD-1 (white), and DAPI (blue). **(B-D)** $CD3^+$ T cell infiltration was analyzed using QuPath. **(B)** Heat maps show that maPYX-201 promoted T cell infiltration from the tumor edge by the sixth day. **(C)** Quantification confirmed greater T cell infiltration deeper into maPYX-201-treated tumors relative to the saline group. (D) $PD\text{-}1^+$ T cells were more densely distributed per unit area in maPYX-201-treated tumors.

Combining maPYX-201 with anti-PD1 results in superior tumor clearance

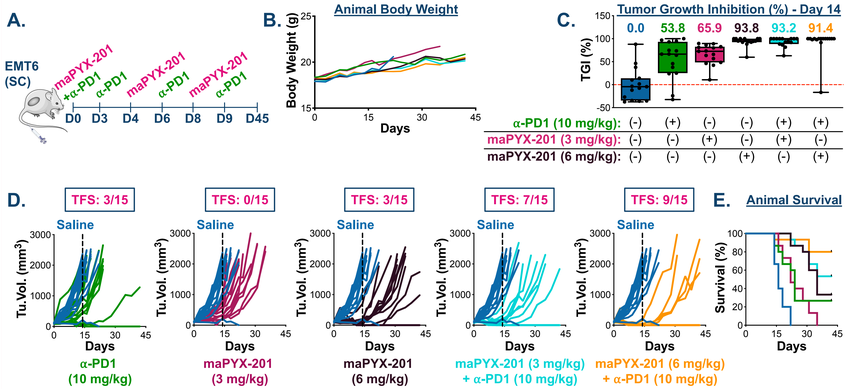


Figure 4: maPYX-201 and anti-PD1 combination therapy strongly inhibits tumor growth, enhances clearance, and improves survival. (A) EMT6 tumor-bearing mice were treated with saline, maPYX-201 (Q4Dx3), anti-mouse PD1 (RPM1-14; Q3Dx4), or the combination. **(B)** All treatments were well tolerated. **(C)** Anti-PD1 alone provided moderate tumor growth inhibition, while maPYX-201 monotherapy was more effective. Combination therapy with 3 mg/kg maPYX-201 further enhanced inhibition, whereas 6 mg/kg maPYX-201 alone achieved a similarly high level. **(D-E)** Spider and survival plots showed that combination therapy provided the greatest tumor growth inhibition and resulted in the highest number of tumor-free surviving (TFS) mice, outperforming both monotherapies.

maPYX-201 elicits potent immune memory that prevents tumor recurrence

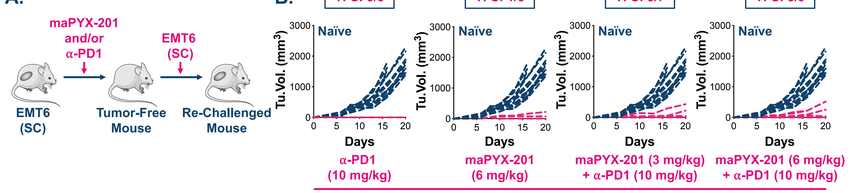


Figure 5: maPYX-201 induces durable immunological memory, with anti-PD1 enhancing tumor clearance. (A) Tumor-free mice from the combination study above were rechallenged with 0.3 × 10^6 EMT6 cells in the opposite flank without additional treatment, alongside age-matched naïve Balb/c mice as controls. All previously treated groups restricted tumor outgrowth, including monotherapy maPYX-201, indicating immunological memory. **(B)** Spider plots show greater tumor control in rechallenged mice, with the maPYX-201+anti-PD1 combination yielding the most tumor-free survivors.

Conclusions

- **Monotherapy with maPYX-201 results in dose-dependent growth inhibition of EDB+FN-expressing EMT6 tumors and is well tolerated.**
- **This anti-tumor activity drives immunogenic cell death and converts T cell-excluded EMT6 tumors into immune-infiltrated, "hot" tumors.**
- **Combining maPYX-201 with anti-mouse PD1 immunotherapy enhances tumor clearance and generates durable immunological memory that protects against tumor recurrence.**
- **Together, these findings support the clinical development of PYX-201 (Micvotabart Pelidotin) in combination with pembrolizumab for the treatment of difficult-to-cure cancers (NCT06795412).**

References

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