

Micvotabart pelidotin induces immunogenic cell death markers and activates tumor immune cells in pre-clinical studies

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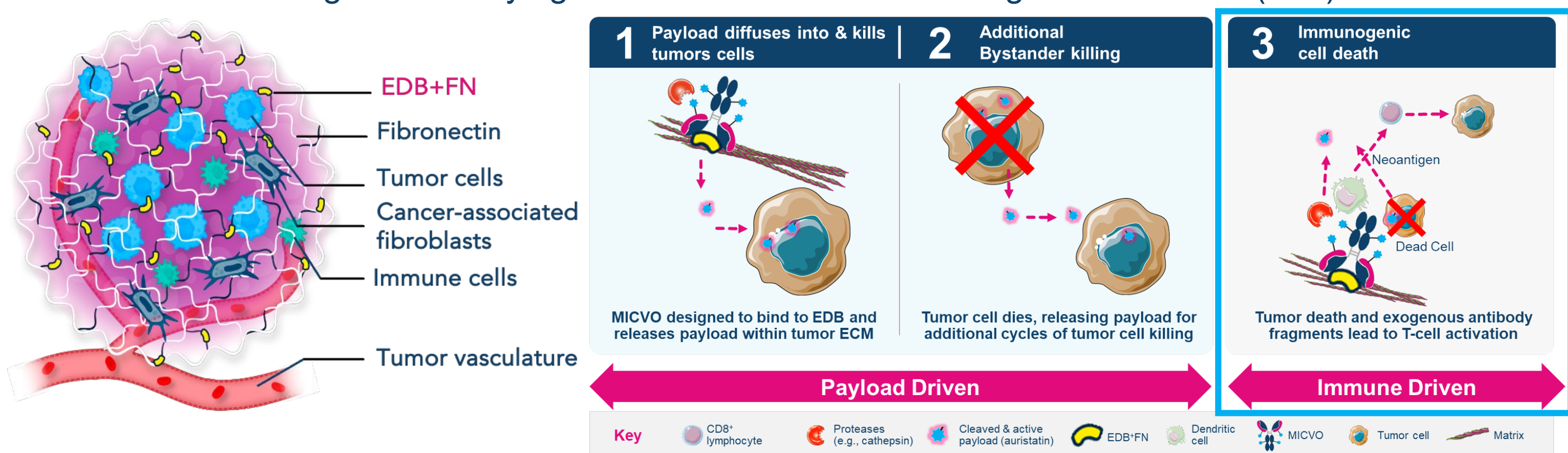
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Abstract: A112

Background

- Micvotabart pelidotin (MICVO, aka PYX-201) is a first-in-concept antibody–drug conjugate (ADC) targeting extradomain-B of fibronectin (EDB+FN), a non-cellular structural component within the tumor extracellular matrix that is highly expressed in tumors compared to normal adult tissues (1).
- MICVO is designed to achieve anti-tumor activity via three mechanisms of action: 1) the cytotoxic, cell-permeable Auristatin-0101 payload directly kills tumor cells through disruption of microtubule formation, 2) the payload promotes additional tumor cell killing via the bystander effect, and 3) release of neoantigens from dying tumor cells induces immunogenic cell death (ICD).



- Recent studies have demonstrated that a mouse analog of MICVO enhanced T cell infiltration in EMT6 tumors and induced strong tumor regression when combined with anti-PD1 (2), supporting clinical development of MICVO as a monotherapy (NCT05720117) and in combination with pembrolizumab (NCT06795412).
- ICD is a type of regulated cell death induced by cancer treatments – including ADCs – that elicits an antigen-specific, adaptive immune response through the release of damage-associated molecular patterns (DAMPs) and tumor antigens, contributing to anti-tumor activity, sensitizing cancers to PD-1/PD-L1 targeting and potentially leading to immunological memory (3).
- The objective of this poster is to characterize MICVO's mechanism of inducing ICD in both in vitro and in vivo settings.**

Methods

- The expression level of DAMP proteins were evaluated after A) treatment with MICVO or a non-targeted ADC (NT-ADC) directly on Caki2 (EDB+FN positive) and HT-29 (EDB+FN negative) cancer cells, or B) treatment with MICVO that was pre-incubated with recombinant cathepsin B at 37°C for 2 hours. Secreted HMGB1, normalized to viability, and extracellular ATP (eATP) were measured from cell supernatants using plate-based assays at day 3 post-treatment. Surface calreticulin (CALR) expression was measured by flow cytometry by gating on caspase 3/7 negative cells at day 1 and 2 post-treatment.
- A co-culture assay using Caki2 cancer cells and non-polarized human macrophages was established to assess MICVO-induced ICD mechanism. Caki2 cells were pre-treated with MICVO or a NT-ADC for 24 hours and then co-cultured with M0 macrophages for 5 days. The phenotype of the macrophages was evaluated by quantifying the expression of different surface markers by flow cytometry after treatment, gating on CD45 to separate cells populations.
- An in vivo study was conducted to analyze the activation of infiltrated T cells in the triple-negative breast cancer (TNBC) syngeneic EMT6 tumor model post-treatment. Immunocompetent mice bearing subcutaneous EMT6 tumors were dosed with 3 mg/kg of a mouse analog of MICVO (maMICVO) (Q4Dx3) or saline buffer, collected after 10 days, enzymatically digested tumors and stained with a T cell flow cytometry panel to measure expression level of activation markers.

MICVO induces early markers of ICD in vitro

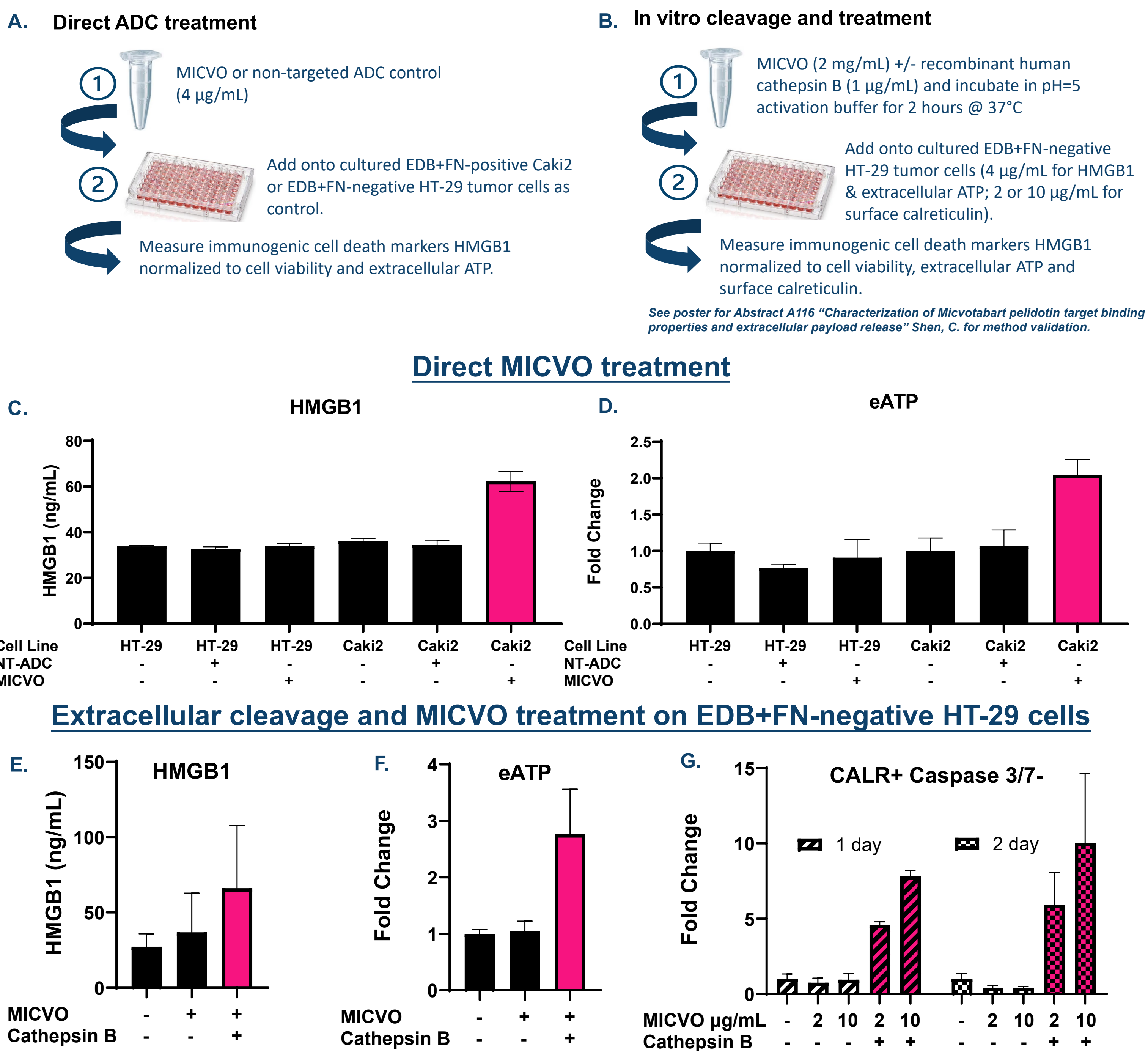


Figure 1. MICVO induces secretion of HMGB1 and eATP and surface expression of calreticulin on cancer cell lines. Expression of early ICD markers were measured after (A) treatment with MICVO or a NT-ADC directly on cancer cells, or (B) treatment with MICVO that was pre-incubated with recombinant human cathepsin B to simulate extracellular proteolytic cleavage of the linker. (C&D) Direct MICVO treatment (described in A) induced secretion of HMGB1 and eATP only on EDB+FN positive Caki2 cancer cells suggesting the effect in vitro is dependent on EDB+FN surface expression of cancer cells. (E&F) Treatment of MICVO, pre-incubated with recombinant cathepsin B to simulate extracellular proteolytic cleavage (described in B) induced secretion of HMGB1 and eATP on EDB+FN negative HT-29 cancer cells. (G) In addition, treatment of MICVO pre-incubated with recombinant cathepsin B also induces HT-29 surface expression of CALR that is normally localized intracellularly. Cells were gated on negative caspase 3/7 staining to assure they were not undergoing apoptosis. These data supports the early induction of ICD by MICVO treatment in vitro. n=2 independent experiments for each assay.

MICVO-induced cell death activates macrophages in vitro

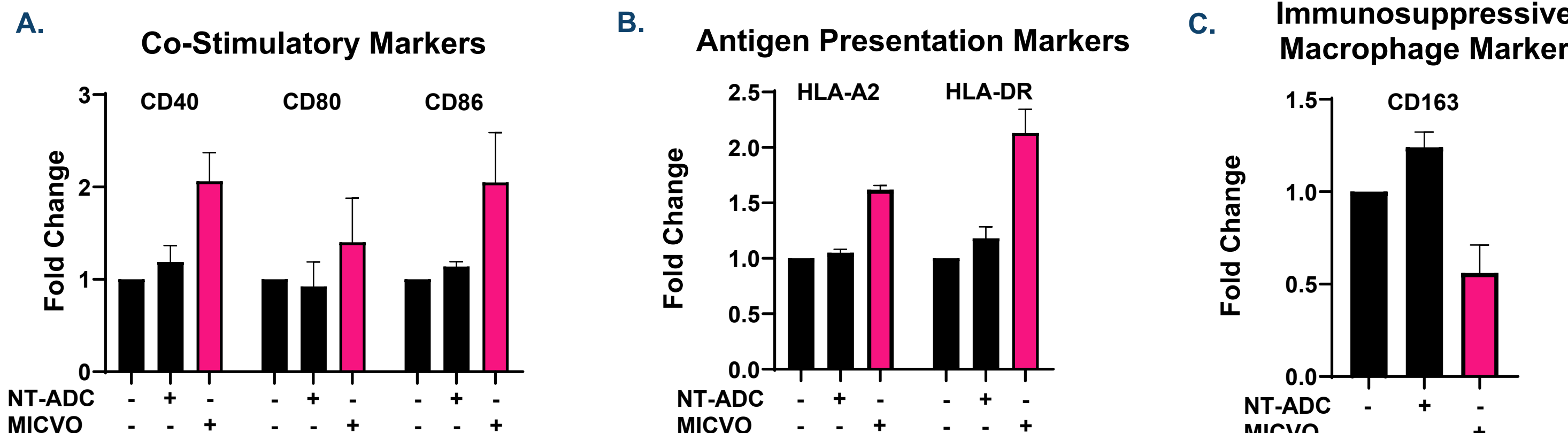


Figure 2. MICVO causes M0 macrophages to have a more activated, antigen presentation-like phenotype. Macrophage maturation markers were measured via flow cytometry, and the fold change over the untreated condition was calculated. After a 5-day co-culture with Caki2 cells directly pre-treated with MICVO, (A) co-stimulatory and (B) antigen presentation markers were upregulated while (C) CD163 – an immunosuppressive, M2 macrophage marker – was downregulated. This data indicates MICVO induces an M1-like macrophage state. n=3 donors.

maMICVO aids infiltration of activated T cells in vivo

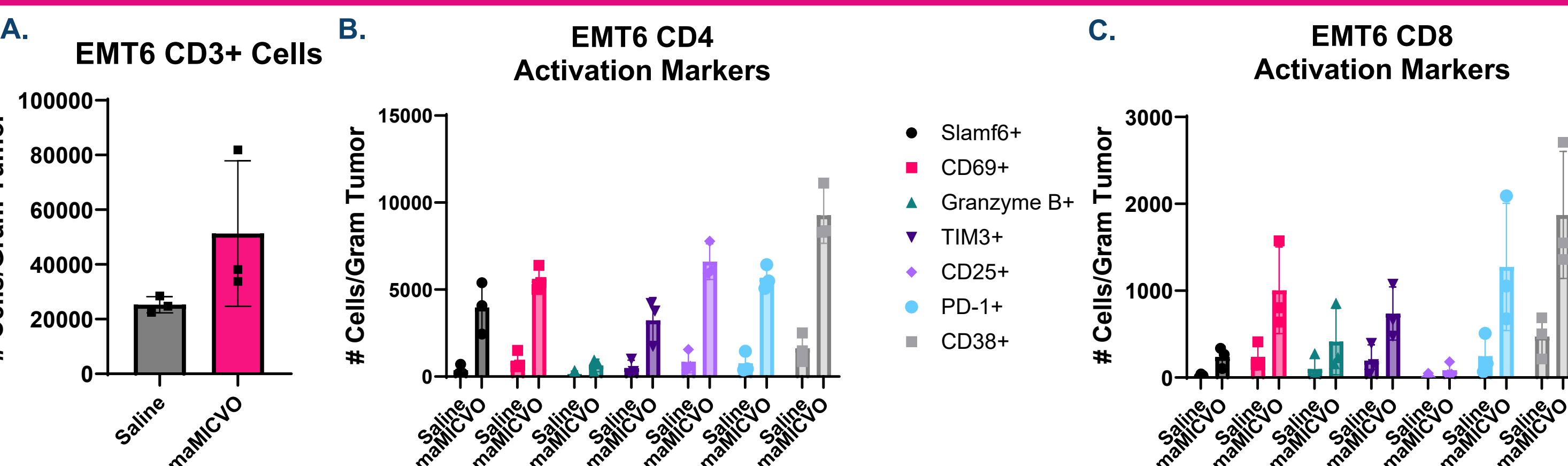


Figure 3. Mouse analog of MICVO (maMICVO) causes T cell infiltration and activation in EMT6 tumors in vivo. (A) More CD3+ T cells per gram of tumors are detected by flow cytometry after maMICVO treatment, validating maMICVO induces T cell infiltration in EMT6 tumors. (B&C) Analysis of mouse T cell activation markers on CD4 and CD8 T cells show maMICVO treatment induces infiltration of activated T cells. n=3 mice per condition.

Conclusions and References

- In vitro, MICVO stimulates early ICD markers on EDB+FN-positive cancer cells as well as via extracellular cleavage on EDB+FN-negative cancer cells. In addition, MICVO-induced cell death activates human macrophages.
- In vivo, maMICVO induced infiltration of activated T cells in the TNBC syngeneic EMT6 mouse tumor model.
- Together, these findings demonstrate MICVO-induced immunogenic cell death is contributing to the overall anti-tumor activity of the ADC, in addition to payload-driven cancer cell killing.
- Future studies will further characterize immune cell populations after MICVO treatment.
- These data provide further rationale for the on-going clinical evaluation of MICVO as a monotherapy (NCT05720117) and in combination with pembrolizumab (NCT06795412).

(1) Lewandowski S. et al., *Cancer Res.* 2024 Mar;84(6):390(10).
(2) Rodriguez A. B. et al., *Cancer Res.* 2025 Apr; 85(8_Supplement_1):3137.

(3) Kepp O. et al., *Oncoimmunology* 2019 Jul 22;8(10).
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