ACTM-838, a Novel Immunotherapy that Enriches in Solid Tumors after IV Dosing and Comprehensively Reverses the Immunosuppressive TME to Promote Durable Anti-tumor Immunity

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Background

- ACTM-838 is a genetically engineered bacterial immunotherapy that encodes an engineered IL-15 (IL-15plex) and a constitutively active STING variant (eSTING). The ACTM-838 strain is a highly modified, attenuated S. Typhimurium that lacks several major inflammatory and immunogenic components on the surface of the microbe and is designed to naturally and specifically enrich in the tumor microenvironment (TME) via (1) obligatory nutrient dependency for adenosine pathway metabolites for extracellular proliferation and (2) intracellular uptake by phagocytic APCs for payload delivery, both of which are highly elevated in the immunosuppressive TME.
- Human macrophage uptake of labeled ACTM-838 led to acidic lysosomes and nuclear delivery with a concomitant increase in expression of encoded IL-15plex and eSTING payloads such as IL-15, IRF3 reporter activity and IFN α/β secretion, respectively (*Udyavar et.al SITC 2022*).
- ACTM-838 treated tumors exhibited a significant increase in human IL-15plex protein payload. ACTM-838 as a single agent induced profound immune reprogramming and remodeling of the TME over time through increased infiltration and activation of myeloid, NK, B and cytolytic CD8 T cells, MHC class I and II, antigen presentation as well as decreased Treg, PD1$^+$Lag3$^+$ exhausted T cells, and a remodeled stromal signature, as assessed by bulk RNAseq, and confirmed by flow cytometry (*Udyavar et.al SITC 2022*).
- Synergistic anti-tumor activity was observed in multiple tumor models when ACTM-838 was dosed in combination with anti-PD1, safely inducing complete durable memory responses (*Udyavar et.al SITC 2022*).

Biodistribution, Tumor-Specific Payload Delivery and Cellular Specificity of ACTM-838

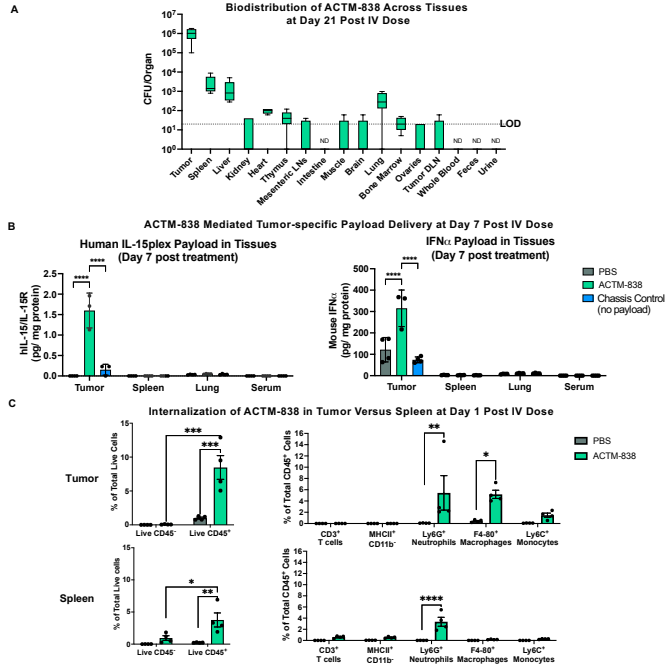

Figure 1. ACTM-838 biodistribution, cellular specificity and tumor-specific payload delivery post single IV dose in EMT6 tumor-bearing Balb/c mice. (A) ACTM-838 biodistribution on D21 post-treatment a single IV dose (3.7e7 CFU/mouse) showed tumor enrichment compared to other healthy tissues. **(B)** Human IL-15plex and IFNα (eSTING target) are detected only in tumor and not in other healthy tissues at D7 post-treatment, suggesting tumor-specific payload delivery. **(C)** Only CD45$^+$ immune cells including the phagocytic APCs (monocytes, neutrophils, DCs and macrophages) internalized ACTM-838 in TME and neutrophils in spleen, suggesting cell type uptake specificity.

ACTM-838 Exhibits Durable Anti-tumor Immunity in Syngeneic Breast and Colon Tumor Models

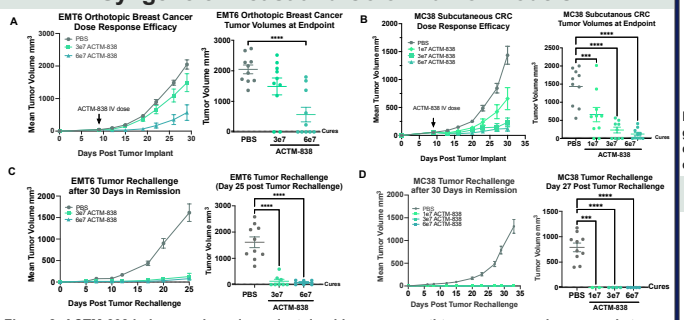

Figure 2. ACTM-838 induces a dose-dependent durable memory anti-tumor response in syngeneic tumor models. (A) EMT6 breast cancer and **(B)** MC38 colon cancer cells were implanted orthotopically or subcutaneously in 6-8 week old female BALB/c or C57BL/6 mice, respectively. Mice received a single IV dose of ACTM-838 when tumors reached 50-100mm^3 volume. ACTM-838 induced a dose dependent anti-tumor effect in both tumor models. **(C-D)** ACTM-838 cured mice rechallenged with 1e5 tumor cells 30 days after initial tumor remission exhibited durable anti-tumor immunity without re-dosing of ACTM-838 in both tumor models.

ACTM-838 Activity in MMTV-PyMT Breast Cancer GEMM

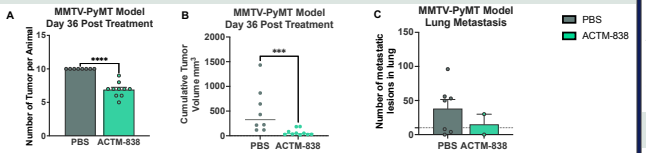

Figure 3. ACTM-838 exhibited anti-tumor responses in MMTV-PyMT GEMM. A single IV dose (6e7 CFU) of ACTM-838 or PBS were given to 8 week old female MMTV-PyMT mice. **(A)** The total number of tumors per animal, **(B)** cumulative tumor volume and **(C)** lung metastasis were significantly reduced in response to ACTM-838 day 36 post treatment.

ACTM-838 Reprograms TME in EMT6 Breast Cancer Model

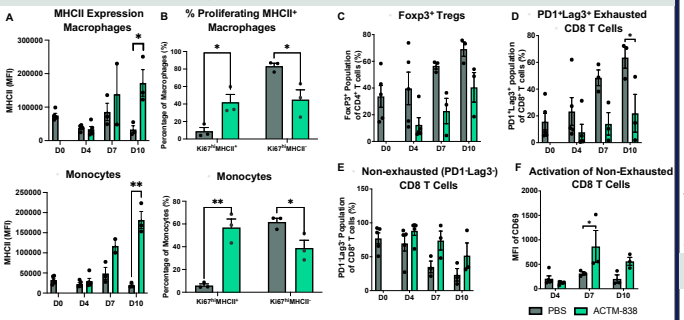

Figure 4. ACTM-838 showed broad myeloid and T cell reprogramming in EMT6 tumors. Immune cells in the tumors were assessed by flow cytometry over time after ACTM-838 (3e7 CFU) treatment. Macrophages and monocytes showed a significant increase in **(A)** MHCII expression over time and **(B)** proliferating Ki67^{high}MHCII$^+$ cells at day 10 post ACTM-838 treatment, **(C)** decreased % of Foxp3$^+$ Treg cells, **(D)** decreased % of PD1$^+$Lag3$^+$ exhausted CD8 T cells, and **(E and F)** increased % and activation of PD1$^-$Lag3$^-$ non-exhausted CD8 T cells over time.

ACTM-838 Reprograms TME in MC38 Colon Tumor Model

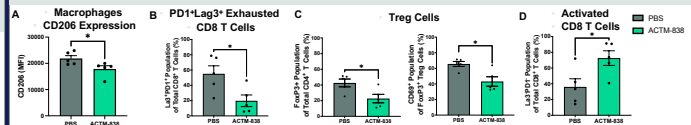

Figure 5. ACTM-838 induces broad TME reprogramming in MC38 tumors. A single dose of ACTM-838 was given IV to mice bearing MC38 tumors. TILs were assessed by flow cytometry on D4 post-treatment. ACTM-838 decreased CD206 expression in **(A)** macrophages, **(B)** exhausted CD8 T cells, **(C)** Treg and **(D)** increased levels of non-exhausted PD1$^-$Lag3$^-$ activated CD8 T cells.

ACTM-838 Reprograms TME in MMTV-PyMT Model

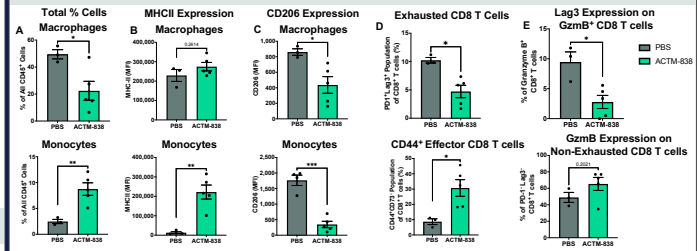

Figure 6. ACTM-838 shows broad TME reprogramming in MMTV-PyMT GEMM model. A single dose of ACTM-838 was given IV to 8-wk-old MMTV-PyMT female mice. Tumors were assessed by flow cytometry on D7 post-treatment. **(A)** ACTM-838 decreased macrophages and increased infiltration of monocytes. Macrophages and monocytes showed an **(B)** increase in MHCII and **(C)** a decrease in CD206 expression. **(D)** ACTM-838 decreased PD1$^+$Lag3$^+$ exhausted CD8$^+$ T cells and increased CD44$^+$ effector CD8$^+$ T cells. **(E)** ACTM-838 reduced the exhausted GzmB$^+$CD8$^+$ T cells and enhanced the GzmB expression on non-exhausted CD8 T$^+$ cells.

ACTM-838 Lowers CD73 Expression on Immune Cells in TME

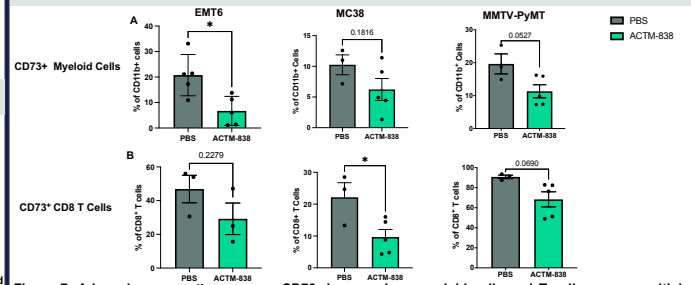

Figure 7. Adenosine generating enzyme CD73 decreased on myeloid cells and T cells across multiple tumor models with ACTM-838 treatment. CD73, an enzyme that generates adenosine from ADP, expressed on immune cells is strongly associated with immunosuppression. ACTM-838 uses adenosine pathway metabolites for its extracellular growth in the TME and thereby reduced proportions of **(A)** CD73$^+$ myeloid and **(B)** CD73$^+$ CD8 T cells across EMT6, MC38 and MMTV-PyMT tumor models.

Summary

- **ACTM-838 shows tumor-specific enrichment where phagocytic APCs take up ACTM-838 and deliver and express IL15plex and eSTING payloads in the TME.**
- **ACTM-838 internalization and payload delivery significantly reprograms the immunosuppressive myeloid cells leading to an increase in antigen presentation (MHCII), costimulatory marker CD86 and reduced phagocytic marker CD206 in monocytes and macrophages.**
- **ACTM-838 activated APCs present tumor antigens to T cells leading to an increase in activated CD8 T cells and a decrease in exhausted CD8 T cells and Tregs, resulting in durable anti-tumor efficacy in multiple syngeneic and GEMM tumor models.**