ACTM-838, A MICROBIAL-BASED IMMUNOTHERAPY THAT ENRICHES IN SOLID TUMORS AFTER IV DOSING, REVERSES THE IMMUNOSUPPRESSIVE TME TO PROMOTE DURABLE ANTI-TUMOR IMMUNITY, ALONE AND IN COMBINATION WITH ANTI-PD1 IN MICE

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**Background**
Effective treatment of metastatic cancers requires reversal of the immunosuppressive tumor microenvironment (TME) and priming a broad repertoire of tumor-specific CD8+ T cells. ACTM-838 is an attenuated, precision genome-engineered, S.Typhimurium-Attenuated Cancer Therapy (STACT) strain carrying a DNA plasmid that encodes payloads consisting of IL-15plex and engineered, constitutively active STING (eSTING). ACTM-838 is designed to colonize the TME and deliver payloads to phagocytic APCs, inducing a durable anti-tumor immune response, after IV dosing.

**Methods**
STACT was developed through genome editing of the parental strain, VNP20009. Single payload (IL-15plex or eSTING) STACT strains and ACTM-838 were bactofected into immune cell lines and primary immune cells. Uptake, payload expression and activity were measured in vitro using ELISAs, MSD, and flow cytometry. ACTM-838 was evaluated in multiple murine tumor models for efficacy as a monotherapy or in combination with anti-PD1 antibodies. Modulation of immune responses in the TME and payload effects were assessed using IHC, RNAseq, flow cytometry and ELISA. ACTM-838 tolerability studies were performed in NHPs and mice.

**Results**
Expression of encoded IL-15plex and eSTING payloads led to IFN-β expression and IL-15 secretion in cell lines and primary M2 macrophages. Furthermore, primary human M2 macrophages polarized toward a novel, co-stimulatory and phagocytic M1/M2 hybrid phenotype. ACTM-838 preferentially colonizes tumors in mice upon IV administration and is well tolerated in NHPs, with enhanced safety compared to VNP20009 in mice. ACTM-838 is selectively taken up by phagocytic APCs in vitro and by tumor-resident APCs in vivo. ACTM-838 treatment showed dose- and payload-dependent anti-tumor efficacy in an anti-PD1 refractory, myeloid rich and T cell excluded, orthotopic EMT6 mouse model as a single agent, and induced a durable anti-tumor CD8+ T cell-dependent memory response upon tumor re-challenge. ACTM-838 induced profound immune reprogramming and remodeling of the TME through increased myeloid cell activation and CD8+ T cell infiltration. Synergistic combination anti-tumor activity was observed when dosed with anti-PD1.

**Conclusions**
ACTM-838 delivers IL-15plex + eSTING payloads to phagocytic APCs in the TME after systemic administration, leading to potent immune reprogramming. Indeed, myeloid cell repolarization, T-cell activation and recruitment promotes durable anti-tumor efficacy as a monotherapy and in combination with anti-PD1. IV-delivered ACTM-838 possesses a compelling safety profile in mice and primates, and is currently in IND-enabling, preclinical development.

**Ethics Approval**
All animals were used according to protocols approved by an Institutional Animal Care and Use Committee and maintained in specific pathogen-free conditions in a AAA-LAC accredited barrier facility.