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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Abstract

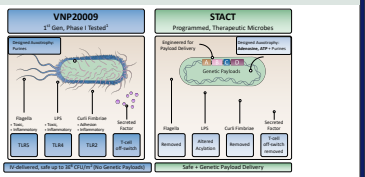
BACKGROUND
Effective treatment of metastatic cancers requires reversal of the immunosuppressive tumor microenvironment (TME) and priming a broad repertoire of tumor-specific CD8+ T cells. ACTM-838 is an attenuated, precision genome-engineered, S.Typhimurium-Attenuated Cancer Therapy (STACT) strain carrying a DNA plasmid that encodes the payloads IL-15 complex (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 cell lines and primary immune cells. Uptake, payload expression and activity were measured in vitro using ELISA, 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 RNAseq, flow cytometry and ELISA.

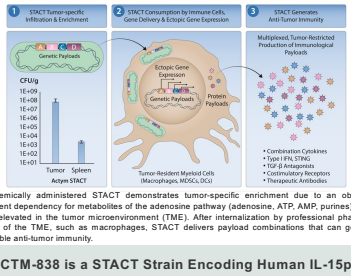
RESULTS
Expression of encoded IL-15plex and eSTING payloads led to IL-15 secretion and IFN-β expression, respectively in cell lines and primary M2 macrophages. STACT preferentially colonizes tumors in mice upon IV administration. Primary human macrophages polarized toward a novel, co-stimulatory and phagocytic M1/M2 dual phenotype in response to ACTM-838. ACTM-838 is selectively internalized 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 breast mouse tumor model as a single agent and induced a durable anti-tumor memory response upon tumor re-challenge. ACTM-838 induced profound immune reprogramming and remodeling of the TME through increased myeloid and CD8+ T cell infiltration and activation. Synergistic anti-tumor activity was observed in multiple tumor models when dosed in combination with anti-PD1 antibody.

CONCLUSION:
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, and is currently in IND-enabling, preclinical development.

STACT: S. Typhimurium (Attenuated) Cancer Therapy a Microbial-Based, Tumor-Specific Delivery Platform

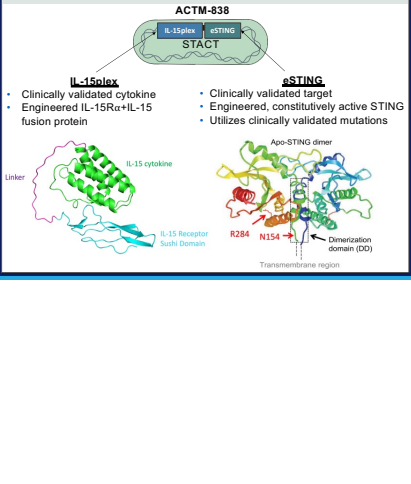


STACT has been genetically edited to remove highly inflammatory surface components (flagella, curli fimbriae, altered LPS acylation), and engineered for tumor-specific payload delivery.



Systemically administered STACT demonstrates tumor-specific enrichment due to an obligatory nutrient dependency for metabolites of the adenosine pathway (adenosine, ATP, AMP, purines), which are elevated in the tumor microenvironment (TME). After internalization by professional phagocytic cells of the TME, such as macrophages, STACT delivers payload combinations that can generate durable anti-tumor immunity.

ACTM-838 is a STACT Strain Encoding Human IL-15plex and an Engineered Human STING Variant (eSTING)



Expression of ACTM-838 Encoded IL-15plex and eSTING Payloads Leads to High IL-15 Secretion and IFN-β Expression

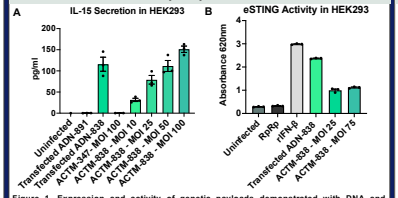


Figure 1. Expression and activity of genetic payloads demonstrated with DNA and ACTM-838 transfection in HEK293 Dual™ STING Null cells
Cells were transfected with DNA plasmids encoding IL-15plex+eSTING ADN-838, control plasmid ADN-891, a control STACT, ACTM-347 or different MOI of ACTM-838. After 48 hours, supernatants were measured for **(A)** IL-15 by ELISA or **(B)** ISG-SEAP reporter activity. IL-15plex protein was detectable in cells transfected with plasmid ADN-838 or STACT ACTM-838 but not negative control (ACTM-347). ACTM-838 eSTING payload bioactivity was comparable to recombinant IFNβ and transfected ADN-838 plasmid in STING Null cells while no activity was detected for control RpRp STING agonist or control plasmid, ADN-891 (not shown).

Selective Internalization of ACTM-838 by Primary Phagocytic M2 Macrophages

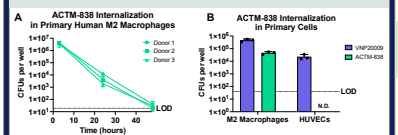


Figure 2. Uptake of ACTM-838 in primary human cells is specific to phagocytic cells
(A) Primary human M2 macrophages were bactofected with ACTM-838 (MOI 75) and bacteria colony forming units (CFU) were enumerated by spot plating over time. **(B)** Human primary M2 macrophages and umbilical vein endothelial cells (HUVECs) were bactofected with a MOI 50 of ACTM-838 or the parental strain VNP20009. CFUs were enumerated. ACTM-838 uptake is specific to phagocytic M2 macrophages. ACTM-838 is not internalized by HUVECs while VNP20009 is internalized by both HUVECs and M2 macrophages.

ACTM-838 is Internalized and Trafficked to Acidic Lysosomes and Nuclei in Human Macrophages

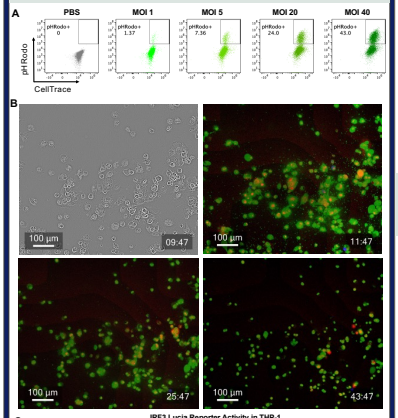


Figure 3. ACTM-838 uptake leads to lysosomal and nuclear localization in THP-1 macrophages
(A) THP-1 Dual (IRF3 Lucia and NF-κB SEAP reporter) cells were treated with PMA for 24h and rested for 3 days to differentiate into macrophages. ACTM-838 was labeled with pHRodo, a pH sensitive dye that fluoresces upon lysosomal uptake and Celltrace Violet, a dye that binds free amines in STACT. Dual labeled ACTM-838 were then added to THP-1 cells at different MOI. After 90-minutes, THP-1 cells were washed and treated with gentamicin to kill non-internalized ACTM-838. THP-1 cells were then harvested for flow analysis to measure pHRodo and Celltrace. **(B)** THP-1 nuclei were stained with DNA dye DRAQ5 (red) before bactofection with ACTM-838 pre-labeled with bacterial DNA dye (bright green) at MOI 60. Timelapse images were acquired using the CellCyteX™ imager immediately after bactofection. ACTM-838 enters THP-1 macrophage cytoplasm shown by intracellular green staining and lysosomes via phagocytosis (pHRodo+ in (A)) and traffics to the nucleus over time indicated by yellow/orange nuclei. **(C)** THP-1 were treated with different MOI of ACTM-838 or cGAMP (positive control), and eSTING-induced IRF3 luciferase reporter activity was measured at 48 hours, showing an MOI dependent increase in STING reporter activity.

ACTM-838 Delivers eSTING Payload in STING KO BMDMs and Induces Activation to a Dual M1/M2 State

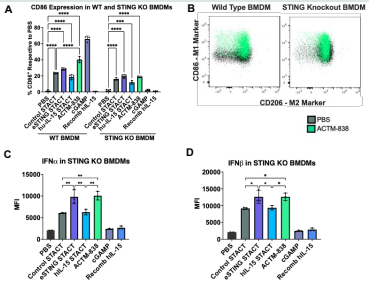


Figure 4. eSTING payload delivery in ACTM-838 treated primary BMDMs
Unpolarized (M0) primary mouse bone marrow derived macrophages (BMDMs) were bactofected at an MOI of 50. **(A-B)** After 24 hours, cells were assessed for phenotypic markers CD86 (co-stimulation) and CD206 (phagocytosis) by flow cytometry. ACTM-838 induces a dual M1/M2 state. **(C-D)** After 48 hours cytokines in the supernatant were assessed by Biolegend® Legendplex™. IFNα and IFNβ secretion are downstream of exogenous eSTING-IRF3 signaling mediated by ACTM-838.

ACTM-838 Stimulates Dual M1/M2 Phenotypic and Functional Activities in Primary Human MDMs

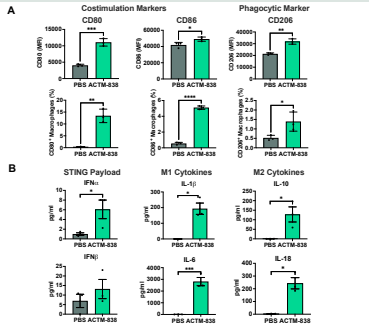


Figure 5. ACTM-838 induces phenotypic and functional changes in primary human monocyte derived macrophages (MDMs) to a dual M1/M2 state
Primary human M0 macrophages were bactofected at MOI=40 of ACTM-838. After 48 hours, **(A)** phenotypic markers were assessed by flow cytometry and **(B)** cytokines in supernatants were measured by MSD.

Single Agent ACTM-838 Activity in Syngeneic Breast and Colon Tumor Models with Durable Anti-tumor Immunity Upon Tumor Rechallenge

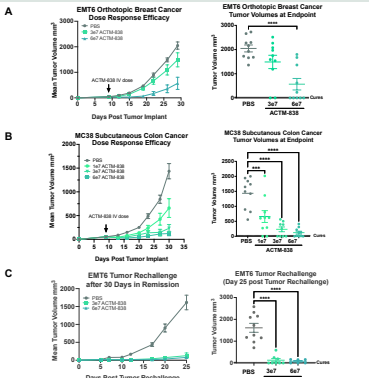


Figure 6. ACTM-838 induces a dose-dependent anti-tumor response in EMT6 and MC38 tumor models
(A) EMT6 breast cancer and **(B)** MC38 colon adenocarcinoma cells were implanted orthotopically in mammary fat pad of 6-8 week old female BALB/c or subcutaneously in the right flanks of 6-8 week old female C57BL/6 mice, respectively. When tumors reached 50-75mm³ volume, mice received a single IV dose of ACTM-838 (1e7, 3e7 or 6e7 CFU/mouse). **(A-B)** Both tumor models exhibited a strong dose dependent anti-tumor response. **(C)** ACTM-838 cured BALB/c mice were rechallenged with freshly implanted 1e5 EMT6 cells in the contralateral orthotopic mammary fat pad 30 days after initial tumor remission showed high tumor growth inhibition and a significant increase in complete responses (CR) compared to treatment naïve EMT6 tumors. This demonstrates strong immunological memory in ACTM-838 treated animals.

ACTM-838 Induces Comprehensive and Broad Reprogramming of the Immunosuppressive TME Towards an Activated Anti-tumor Immunity Profile

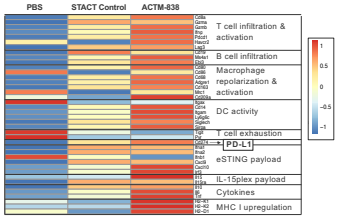


Figure 7. ACTM-838 reverses the immunosuppressive TME in EMT6 breast tumors
1e5 EMT6 breast tumor cells were implanted orthotopically in the mammary fat pad of 6-8 week old female BALB/c mice. When tumor size reached 50-75mm³, mice were treated with a single IV dose of either vehicle (PBS, N=10), STACT control (STST-347, an inactive version of IL-15 and eSTING, N=5 each at 6e7 and 9e7 CFU/mouse) and ACTM-838 (N=5 each at 6e7 or 9e7 CFU/mouse). Day 4 after treatment, bulk RNAseq was performed from extracted tumors. Shown is the average expression of genes across all mice in a given treatment group. ACTM-838 upregulates the gene expression profiles of T cell, B cell and myeloid cell infiltration and activation, T cell effector function as well as eSTING payload targets, MHC I antigen presentation, and pro-inflammatory cytokines.

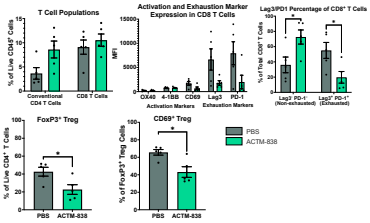


Figure 8. ACTM-838 reprograms T cells in MC38 colon tumors
5e5 MC38 colon tumor cells were implanted s.c. in the right flank of 6-8 week old female C57/B6 mice. When tumors reached 50-75mm³, mice were administered a single IV dose of 3e7 CFU/mouse of ACTM-838. After 4 days, tumors were processed and analyzed by flow cytometry. T cell infiltration increases with ACTM-838, along with a downregulation of exhaustion markers (PD1, LAG3) on CD8 T cells as well as total and CD69+ immunosuppressive Foxp3+ CD4 Treg cells. Similar changes were observed in EMT6 tumors.

Synergistic Activity of ACTM-838 in Combination with Anti-PD1 in Two Independent Syngeneic Tumor Models

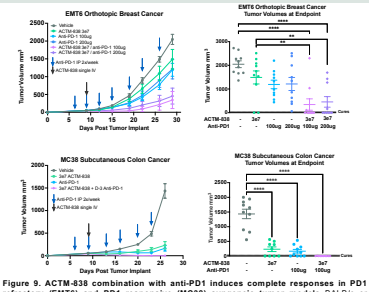


Figure 9. ACTM-838 combination with anti-PD1 induces complete responses in PD1 refractory (EMT6) and PD1 responsive (MC38) syngeneic tumor models BALB/c or C57BL/6 mice were implanted orthotopically or s.c. with 1e5 (EMT6 – top panels) or 5e5 (MC38 – bottom panels) cells, respectively, and allowed to reach 50-75mm³ prior to treatment with a single IV dose of ACTM-838 (3e7 CFU/mouse). The anti-PD-1 i.p. treatment was initiated 2-3 days prior to ACTM-838 and continued 2x/week throughout the study. ACTM-838 dosed at 3e7 CFU per mouse in combination with anti-PD1 in EMT6 orthotopic breast cancer and MC38 s.c. colon cancer models show a synergistic effect. The regimen was well-tolerated in both models.

Summary and Conclusions

- ACTM-838 is a STACT that encodes 2 genetic payloads: IL-15plex (a single-chain IL-15Rα sushi domain + IL-15 fusion protein) and an engineered form of human STING (eSTING). Each payload demonstrated high-level expression in HEK-293 cells.
- ACTM-838 is selectively internalized by primary human macrophages. ACTM-838 was not internalized by endothelial cells. In a human macrophage cell line (THP-1), uptake into acidic lysosomes and nuclear delivery of labeled ACTM-838 was observed.
- ACTM-838 delivers eSTING payload in STING knockout murine BMDMs and induces significantly higher Type I IFN production over a STACT control strain.
- Phagocytosis by ACTM-838 induces a preferred dual M1/M2 dual phenotype in primary human macrophages.
- A single IV dose of ACTM-838 shows dose dependent and durable efficacy in two independent syngeneic breast and colon tumor models. Sustained long term anti-tumor immunity upon tumor rechallenge in cured animals was demonstrated.
- Significant tumor microenvironment reprogramming is induced by ACTM-838 as a single agent in both EMT6 and MC38 tumor models.
- Synergistic potency of ACTM-838 in combination with an anti-PD1 antibody was observed in two syngeneic tumor models, including the orthotopic, metastatic checkpoint-refractory EMT6 breast tumor model. The combination safely induced complete responses. No significant body weight loss and no death was observed in any treatment regimens at tested doses of ACTM-838 alone or in combination with anti-PD1.