#408 PK/PD Biomarker Analysis to Assess Tumor-Specific Enrichment and Payload Delivery of ACTM-838, a Microbial-Based Immunotherapy

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ABSTRACT

Background

ACTM-838 is a microbial immunotherapy that encodes an engineered IL-15 (IL-15plex) and a constitutively active STING (eSTING). ACTM-838 is a highly modified, attenuated *S. Typhimurium* lacking several major inflammatory components on the microbial surface and designed to naturally and specifically enrich in the TME via auxotrophic dependency on metabolites of the adenosine pathway and purines to achieve tumor-specific payload delivery.

Methods

ACTM-838 uptake, payload expression, and activity were measured using qPCR, MSD, and flow cytometry in human and mouse samples. TME immune responses and payload effects were assessed using single cell RNAseq, flow cytometry, and ELISA.

Results

In EMT6 tumor-bearing mice, a single IV dose of ACTM-838 was able to rapidly distribute and enrich in the TME compared to other tissues and exhibited specific uptake in the phagocytic antigen-presenting cells such as monocytes, macrophages and neutrophils; whereas in liver, spleen and blood, neutrophils showed the highest uptake. This led to a significantly increased expression of human IL-15plex and murine IFN-α in the tumor compared to other tissues. Ex vivo bactofection of EMT6 tumors exhibited a dose-dependent increase in cellular uptake of ACTM-838, maintaining its preferential uptake in the myeloid compartment. ACTM-838 showed a significantly decreased inflammatory cytokine profile compared to parental strain VNP20009.

ACTM-838 showed significantly prolonged anti-tumor efficacy in EMT6 breast cancer, MC38 colon tumor, and MMTV-PyMT GEMM tumor models and exhibited significant reprogramming in the TME as well as peripherally, with a decrease in exhausted T cells and Treg and an increase in activated CD8 T cells and MHCII-high proliferating myeloid cells. In addition, a significant decrease in adenosine-generating enzyme CD73 across myeloid and T cell populations was observed, suggesting reduced immunosuppression. Human MDMs exhibited significantly reduced pro-inflammatory cytokines with high expression of co-stimulatory markers and MHCII with ACTM-838 compared to VNP20009.

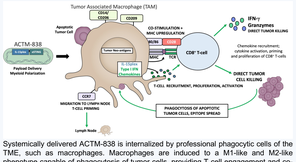
ACTM-838 was stable in human whole blood with minimal complement activation. Serum from healthy donors and cancer patients showed varying levels of anti-salmonella antibodies with minimal neutralization of ACTM-838. Indication prioritization analyses using the TCGA and MET500 datasets identified key indications with high myeloid content as well as adenosine and purine metabolism.

Conclusion

ACTM-838 is a novel immunotherapy delivering IL-15plex + eSTING payloads to phagocytic APCs, inducing a durable anti-tumor immune response after IV dosing. IV-delivered ACTM-838 possesses a compelling safety profile in mice and primates and is currently entering clinical trials in cancer patients in Australia.

BACKGROUND

ACTM-838 Induces a Costimulatory & Migratory (M1-Like) and Phagocytic (M2-Like) Anti-Tumor Phenotype in TAMs

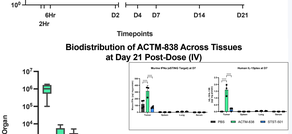


Systemically delivered ACTM-838 is internalized by professional phagocytic cells of the TME, such as macrophages. Macrophages are induced to a M1-like and M2-like phenotype capable of phagocytosis of tumor cells, providing T cell engagement and co-stimulation, and expressing ACTM-838-encoded factors (eSTING/IL-15plex) to support anti-tumor immunity[1,2].

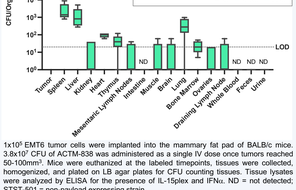
DRUG STABILITY AND PHARMACOKINETICS

ACTM-838 Exhibits Tumor-Specific Enrichment and Persistence Over Time Compared to Other Tissues

Pharmacokinetics of ACTM-838



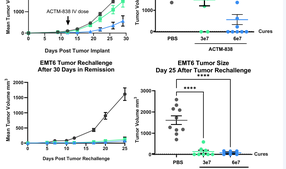
Biodistribution of ACTM-838 Across Tissues at Day 21 Post-Dose (IV)



1x10[5] EMT6 tumor cells were implanted into the mammary fat pad of BALB/c mice. 3.8x10[7] CFU of ACTM-838 was administered as a single IV dose once tumors reached 50-100mm[3]. Mice were euthanized at the labeled timepoints, tissues were collected, homogenized, and plated on LB agar plates for CFU counting tissues. Tissue lysates were analyzed by ELISA for the presence of IL-15plex and IFNα. ND = not detected; STST-501 = non-payload expressing strain.

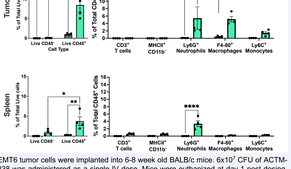
EFFICACY & PAYLOAD DELIVERY IN SYNGENIC MOUSE MODELS

ACTM-838 Exhibits Durable Anti-Tumor Immunity in Syngeneic Tumor Models



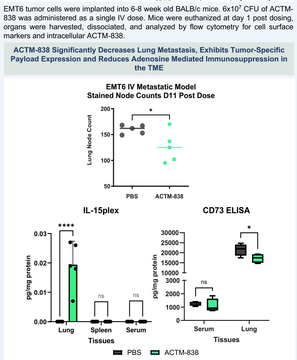
EMT6 breast cancer cells were implanted orthotopically in 6–8-week-old female BALB/c mice. A single IV dose of ACTM-838 administered when tumors reached 50–100 mm[3] volume induced a dose-dependent anti-tumor effect with increasing number of complete cures. Durable anti-tumor immunity was observed when rechallenged with fresh tumor cells in the contralateral mammary fat pad in cured animals after 30 days in remission. Similar results were observed in MC38 colon tumor syngeneic and MMTV-PyMT TNBC GEMM models[1].

ACTM-838 Exhibits Specific Cellular Uptake in Myeloid Cells Across Tissues



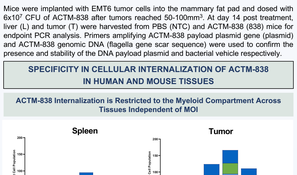
EMT6 tumor cells were implanted into 6-8 week old BALB/c mice. 6x10[5] CFU of ACTM-838 was administered as a single IV dose. Mice were euthanized at day 1 post dosing, organs were harvested, dissociated, and analyzed by flow cytometry for cell surface markers and intracellular ACTM-838.

ACTM-838 Significantly Decreases Lung Metastasis, Exhibits Tumor-Specific Payload Expression and Reduces Adenosine Mediated Immunosuppression in the TME



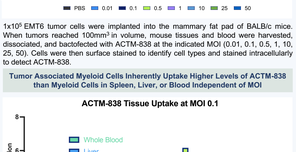
1x10[5] EMT6-M tumor cells were intravenously injected into BALB/c mice. 6x10[7] CFU of ACTM-838 was administered as a single dose 3 days post tumor implantation. Mice were euthanized on day 11 and tissues were analyzed for the presence of lung metastases, soluble IL-15plex and CD73 expression. Similar results were observed in MC38 and MMTV-PyMT GEMM tumor models[1].

ACTM-838 Reprograms the TME to Pro-Inflammatory Phenotype and Delivers the Genetic Payload



1x10[5] EMT6 tumor cells were implanted into the mammary fat pad of BALB/c mice. 6x10[7] CFU of ACTM-838 was administered as a single dose once tumors reached 50-100mm[3] in volume. Mice were euthanized and tumors collected on day 2 and gene expression was analyzed by Nanostring IO360.

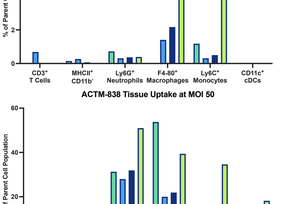
ACTM-838 is Genetically Stable and Payload Encoding Plasmid is Maintained in vivo in EMT6 Tumor-Bearing Mice 14 days Post IV Dosing



Mice were implanted with EMT6 tumor cells into the mammary fat pad and dosed with 6x10[7] CFU of ACTM-838 after tumors reached 50-100mm[3]. At day 14 post treatment, liver (L) and tumor (T) were harvested from PBS (NTC) and ACTM-838 (838) mice for endpoint PCR analysis. Primers amplifying ACTM-838 payload plasmid gene (plasmid) and ACTM-838 genomic DNA (flagella gene scar sequence) were used to confirm the presence and stability of the DNA payload plasmid and bacterial vehicle respectively.

SPECIFICITY IN CELLULAR INTERNALIZATION OF ACTM-838 IN HUMAN AND MOUSE TISSUES

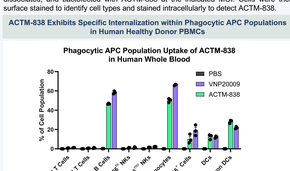
ACTM-838 Internalization is Restricted to the Myeloid Compartment Across Tissues Independent of MOI



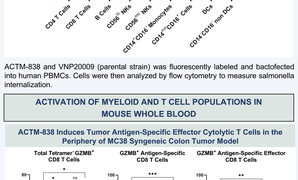
1x10[5] EMT6 tumor cells were implanted into the mammary fat pad of BALB/c mice. When tumors reached 100mm[3] in volume, mouse tissues and blood were harvested, dissociated, and bactofected with ACTM-838 at the indicated MOI (0.01, 0.1, 0.5, 1, 10, 25, 50). Cells were then surface stained to identify cell types and stained intracellularly to detect ACTM-838.

Tumor Associated Myeloid Cells Inherently Uptake Higher Levels of ACTM-838 than Myeloid Cells in Spleen, Liver, or Blood Independent of MOI

ACTM-838 Tissue Uptake at MOI 0.1



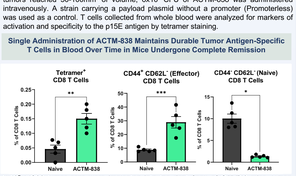
ACTM-838 Tissue Uptake at MOI 50



1x10[5] EMT6 tumor cells were implanted into the mammary fat pad of BALB/c mice. When tumors reached 100mm[3] of volume, mouse tissues and blood were harvested, dissociated, and bactofected with ACTM-838 at the indicated MOI. Cells were then surface stained to identify cell types and stained intracellularly to detect ACTM-838.

ACTM-838 Exhibits Specific Internalization within Phagocytic APC Populations in Human Healthy Donor PBMCs

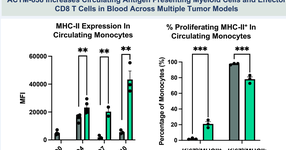
Phagocytic APC Population Uptake of ACTM-838 in Human Whole Blood



ACTM-838 and VNP20009 (parental strain) was fluorescently labeled and bactofected into human PBMCs. Cells were then analyzed by flow cytometry to measure salmonella internalization.

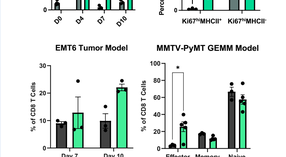
ACTIVATION OF MYELOID AND T CELL POPULATIONS IN MOUSE WHOLE BLOOD

ACTM-838 Induces Tumor Antigen-Specific Effector Cytolytic T Cells in the Periphery of MC38 Syngeneic Colon Tumor Model



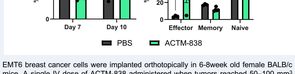
1x10[5] MC38 tumor cells were implanted into the right flank of C57/Bl6 mice. When tumors reached 50-100mm[3] of volume, 6x10[7] CFU of ACTM-838 was administered intravenously. A strain carrying a payload plasmid without a promoter (Promoterless) was used as a control. T cells collected from whole blood were analyzed for markers of activation and specificity to the p15E antigen by tetramer staining.

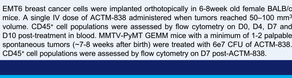
Single Administration of ACTM-838 Maintains Durable Tumor Antigen-Specific T Cells in Blood Over Time in Mice Undergone Complete Remission



1x10[5] MC38 tumor cells were implanted into the right flank of C57/Bl6 mice. When tumors reached 50-100mm[3] of volume, 6x10[7] CFU of ACTM-838 was administered IV. Mice that showed complete tumor remission for over 30 days were used for whole blood collection. T cells from whole blood were analyzed for markers of activation and specificity to the p15E antigen by tetramer staining.

ACTM-838 Increases Circulating Antigen Presenting Myeloid Cells and Effector CD8 T Cells in Blood Across Multiple Tumor Models

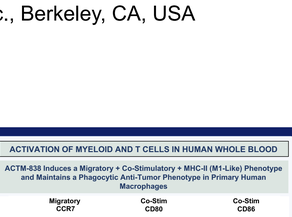




EMT6 breast cancer cells were implanted orthotopically in 6-8week old female BALB/c mice. A single IV dose of ACTM-838 administered when tumors reached 50–100 mm[3] volume. CD45+ cell populations were assessed by flow cytometry on D0, D4, D7 and D10 post-treatment in blood. MMTV-PyMT GEMM mice with a minimum of 1-2 palpable spontaneous tumors (~7-8 weeks after birth) were treated with 6e7 CFU of ACTM-838. CD45+ cell populations were assessed by flow cytometry on D7 post-ACTM-838.

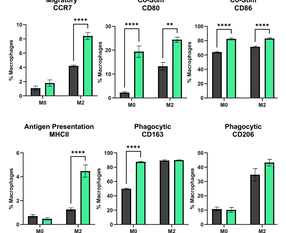
ACTIVATION OF MYELOID AND T CELLS IN HUMAN WHOLE BLOOD

ACTM-838 Induces a Migratory + Co-Stimulatory + MHC-II (M1-Like) Phenotype and Maintains a Phagocytic Anti-Tumor Phenotype in Primary Human Macrophages



Primary human M0 or M2 macrophages were bactofected at MOI=40 of ACTM-838. After 48 hours, phenotypic markers were assessed by flow cytometry.

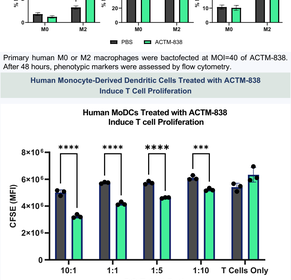
Human Monocyte-Derived Dendritic Cells Treated with ACTM-838 Induce T Cell Proliferation



Human moDCs were bactofected with ACTM-838 (MOI 40) followed by coculture with CFSE labeled T cells at different E:T ratios. At D4, the T cells were harvested and dilution of CFSE via proliferation assessed by flow cytometry.

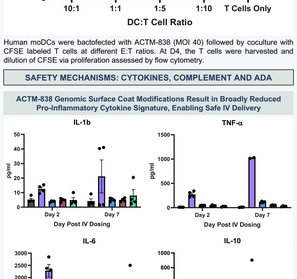
SAFETY MECHANISMS: CYTOKINES, COMPLEMENT AND ADA

ACTM-838 Genomic Surface Coat Modifications Result in Broadly Reduced Pro-Inflammatory Cytokine Signature, Enabling Safe IV Delivery



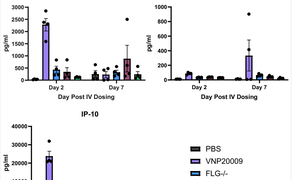
1x10[5] EMT6 tumor cells were implanted into the mammary fat pad of BALB/c mice. Once tumors reached 50-100mm[3], 3x10[7] CFU of VNP20009, a control strain with deleted flagella (FLG-/-), STST-501 (ACTM-838 with no functional payload) or ACTM-838 was administered as a single dose. At D2 and D7 post IV dosing, serum was collected and tested for cytokines by MSD.

ACTM-838 Induces Significantly Lower Inflammatory Cytokines than VNP20009 in Human MDMs



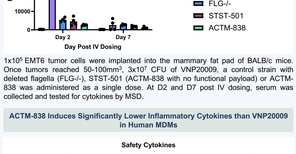
Human monocytes were differentiated into M0, M1 and M2 macrophages using various stimuli. MDMs were bactofected for 1 hour with MOI 40 of ACTM-838 or VNP20009 and treated with gentamycin to remove extracellular bacteria. Cytokines were measured in the media supernatant by MSD at 48 hours post-bactofection. Data shown is M0 macrophages. Similar trends were observed in M2 macrophages.

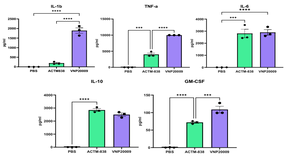
ACTM-838 is Stable in Whole Blood with Significantly Reduced Complement Activation Compared to VNP20009



Human whole blood from healthy donors was incubated at 37 degrees with either PBS, ACTM-838 or VNP20009 for the indicated timepoints. At each timepoint, 100ul of blood was assessed for complement activation by ELISA or plated on LB agar plates for CFU counting.

ACTM-838 is Stable in Presence of Pre-Existing Anti-Salmonella Antibodies in Human Serum

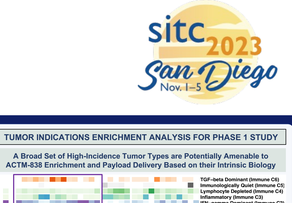




Human serum from healthy donors and cancer patients was incubated with live-dead dye labeled ACTM-838 for 30 minutes. ACTM-838 binding to pre-existing serum anti-salmonella IgG was assessed by flow cytometry using anti-human secondary IgG antibody. Anti-salmonella antibodies in human serum bind VNP-20009 more efficiently than ACTM-838. Minimal impact on ACTM-838 viability was observed by pre-existing anti-salmonella IgG.

TUMOR INDICATIONS ENRICHMENT ANALYSIS FOR PHASE 1 STUDY

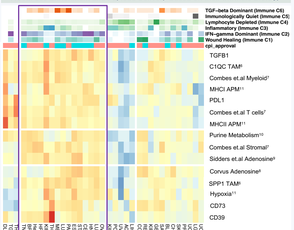
A Broad Set of High-Incidence Tumor Types are Potentially Amenable to ACTM-838 Enrichment and Payload Delivery Based on their Intrinsic Biology



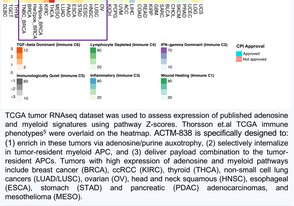
TCGA tumor RNAseq dataset was used to assess expression of published adenosine and myeloid signatures using pathway Z-scores. Thorsson et.al TCGA immune phenotypes[4] were overlaid on the heatmap. ACTM-838 is specifically designed to: (1) enrich in these tumors via adenosine/purine auxotrophy, (2) selectively internalize in tumor-resident myeloid APC, and (3) deliver payload combination to the tumor-resident APCs. Tumors with high expression of adenosine and myeloid pathways include breast cancer (BRCA), ccRCC (KIRC), thyroid (THCA), non-small cell lung cancers (LUAD/LUSC), ovarian (OV), head and neck squamous (HNSC), esophageal (ESCA), stomach (STAD) and pancreatic (PDAC) adenocarcinomas, and mesothelioma (MESO).

Preferred Indications Identified Where Majority of Patients Exhibit Both High ADO Pathway (for ACTM-838 Enrichment) and High Myeloid (for Payload Delivery) Signatures

Adenosine Signature vs. Myeloid Signature Ranking Across TCGA



Ranking Histologies with High Adenosine + High Myeloid Signatures

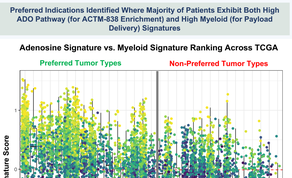


TCGA Tumor RNAseq dataset was used to assess expression of published adenosine and myeloid signatures using pathway Z-scores. Each patient was ranked based on the expression of these scores and patients above 25th percentile of expression were deemed as positive for a given pathway expression. Patients with expression of both adenosine and myeloid pathways above the 25th percentile were assessed for prevalence in a given tumor indication.

ANTIBIOTICS KILL SWITCH FOR ACTM-838

ACTM-838 is Sensitive to Antibiotics in Vivo

Day 14 Post ACTM-1950 (ACTM-838 Surrogate) Treatment in Naive Balb/c Mide



Tissues	% Decrease in ACTM-1950 Colonization with Ciprofloxacin
Whole Blood	98
Spleen	97
Liver	80

Naïve Balb/c mice were administered ACTM-1950 (ACTM-838 surrogate with kanamycin resistance cassette) IV with 3e7 CFU/animal. Ciprofloxacin was dosed 2x daily at 2 mg/mouse for 14 days. Tissues were collected fresh, weighed, processed into tissue homogenates, and plated onto LB agar overnight for CFU counts.

SUMMARY

- ACTM-838 demonstrates
  - Durable anti-tumor efficacy
  - Tumor specific enrichment and persistence
  - Tumor specific payload delivery
  - Cell type specificity restricted to professional APCs
  - Ability to activate the immunosuppressive TME
  - Genomic and plasmid stability in vivo
- ACTM-838 safety
  - Surface coat modifications broadly reduce systemic inflammation and enable safe IV delivery
  - Remains sensitive to frontline antibiotics
  - ADA shows minimal impact on stability
- GLP and non-GLP toxicology in primates
  - Safety observed at all dose levels tested (1x10^{8} - 3x10^{9} CFU/monkey)
  - Transient reversible increase in serum cytokines
  - No adverse findings reported in body weight, hematology, coagulation, and clinical chemistry
  - No shedding detected in urine or feces
- **ACTM-838 is entering Ph1 clinical trials in solid tumors in Q1 2024**

	VNP20009 (Parental Strain)	ACTM-838
Safety	• Genome engineered via msbB for altering LPS acylation[3] • DLT due to high levels of cytokines and persistent bacteremia in Ph1	• Genome engineering to dampen pro-inflammatory effects: Curli fimbriae, flagella, asparaginase activity, LPS acylation, removal of biofilms. • Systemic cytokines significantly reduced in mice, 3e8 CFU well tolerated in NHP GLP tox studies
Tumor Targeting	• Purine auxotrophy enables tumor targeting in mice • Not detected in patient samples until MTD[3]	• Purine + adenosine auxotrophy for tumor specific enrichment and sustained colonization after single IV dose
Cell Type Specificity	• Internalized in epithelial, endothelial and myeloid populations due to flagella[2]	• Internalized only via active phagocytosis by antigen presenting cells in TME
Payload Delivery	• No payloads	• DNA plasmid delivers 2 engineered payloads – IL-15plex and eSTING to the TME • Multiple plasmid copies per bacterial cell allows for efficient payload delivery at lower doses of bacterial vehicle
Efficacy	• No cures were observed in any mouse models, only improvements in survival[2]. • *Prolonged survival in lung metastasis B16 model*[2] • No efficacy observed in melanoma Ph1 trial[3]	• Consistently shows increasing cure rates with durable anti-tumor immunity upon tumor rechallenge in multiple models and significant efficacy in GEMM model • Delayed tumor progression in GEMM, reduced lung metastasis in GEMM and EMT6 metastasis models
TME Re-activation	• Neutrophil infiltration and tumor necrosis observed in mice[2] • Biofilm formation leads to immunosuppressive TME	• Comprehensive tumor re-activation with increased infiltration of T/B/NK cells, activation of effector CD8 T cells, reduction in Treg and Tex, activation and antigen presentation of monocytes and macrophages • Peripheral activation of antigen-specific T cells and myeloid cells

REFERENCES

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