In Vitro and In Vivo Anticancer Effects of D/L-alpha-metyrosine (SM-88), A Novel Metabolism-Based Therapy

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INTRODUCTION

- D/L-alpha-metyrosine (SM-88, racemetyrosine) has displayed broad anticancer activity in patients across 15 different tumor types. While amino acid metabolism has been leveraged in oncology imaging for decades, SM-88 offers a potential novel therapeutic approach to selectively disrupt cancer cells.
- A comprehensive *in vitro* and *in vivo* experimental program is underway to elucidate the mechanism of action and further characterize the anti-cancer effects of SM-88.
- *In vitro* effects on apoptosis, viability, reactive oxygen species induction, autophagy, migration and invasion, cell cycle changes, and protein synthesis are being investigated.
- *In vivo* xenograft models are being used to explore the anti-cancer effects of SM-88 and its potential as an immune modulator.
- This poster provides an update on *in vitro* and *in vivo* data as of May 29, 2020.

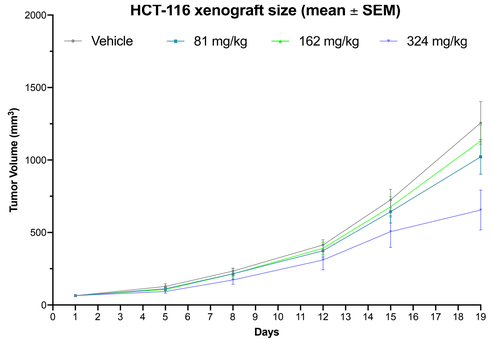
METHODS

- In all *in vitro* cell line experiments, and the Pan02 xenograft model, the SM-88 methyl-ester (SM-88 ME) was used, which has the same active moiety as SM-88, but with improved solubility characteristics. The SM-88 free acid form was used in the HCT-116 xenograft experiment.
- **Autophagy:** Changes in LC3B and p62 expression were investigated using standard Western Blot techniques.
- **ROS Induction:** Following treatment with SM-88, ROS induction was assessed using the CellROX (Thermo-Fisher) flow cytometry assay kit. Gating for ROS+/live cell selection was based on data obtained from negative controls.
- **HCT-116 Xenograft:** Female athymic nude mice were implanted subcutaneously with HCT-116 cells. Once tumors reached 50-100 mm^3, mice were treated with either Vehicle alone, 81 mg/kg/day SM-88, 162 mg/kg/day SM-88, or 324 mg/kg/day SM-88 administered orally (n = 11 per group).
- **Pan02 Xenograft Study:** C57BL/6 mice were treated with either Vehicle alone, 25 mg/kg/day of SM-88 ME via intraperitoneal (IP) injection, or 75 mg/kg/day of SM-88 ME IP (n = 10 per group). Treatments began on Day 0. On Day 4, Pan02 cells were implanted subcutaneously into each mouse.
- **Tumor Immune Profiling:** On Day 34, Pan02 tumors from five randomly selected mice per treatment arm were processed for immune analysis by flow cytometry using a 16 color myeloid and lymphocyte panel following standard protocols.

RESULTS

Oral Administration of SM-88 Reduces Tumor Growth in an HCT-116 Colon Cancer Xenograft Model

Figure 1



Number of HCT-116 Tumor-bearing Mice Alive on Each Treatment Day

Day	1	5	8	12	15	19
Vehicle	11	11	11	11	11	11
81 mg/kg	11	11	11	11	11	11
162 mg/kg	11	11	11	11	11	10
324 mg/kg	11	11	11	11	11	9

- A pilot *in vivo* xenograft experiment was conducted to evaluate the effects of SM-88 in a colon cancer model.
- HCT-116 tumor growth in mice treated with orally administered 324 mg/kg/day SM-88 was significantly reduced compared to those treated with Vehicle alone by Day 19 after treatment initiation ($p < 0.05$).
- No differences in body weight changes were noted between treatment groups.

IP Administration of SM-88 Reduces Tumor Growth in a Pan02 Pancreatic Cancer Syngenic Xenograft Model

Figure 2a

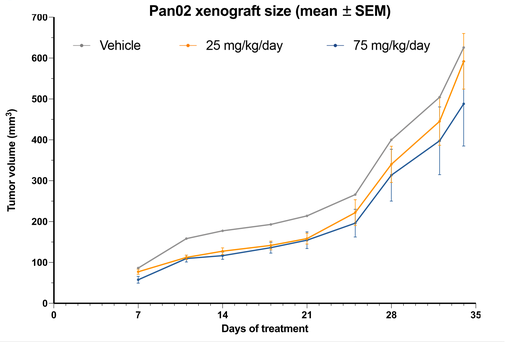
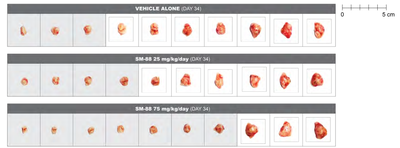


Figure 2b

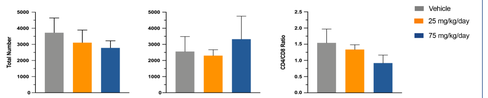


- To further evaluate the anti-cancer effects of SM-88, a second *in vivo* experiment was conducted using a pancreatic cancer model.
- Pan02 tumor growth in mice treated with intraperitoneally administered 75 mg/kg/day SM-88 ME was significantly reduced compared to those treated with Vehicle alone on Day 34 after treatment initiation (Figure 2a).
- All mice (n=30) were alive at end of treatment (Day 34).
- No differences in body weight changes were noted between treatment groups.
- Excised Pan02 tumors (Day 34) from the subcutaneous xenograft model are shown in Figure 2b.
- A higher proportion of mice treated with SM-88 maintained tumors below a volume of 500mm^3 (~2.5% of total body weight), with an indication of dose-related effect.

Alterations to the Pan02 Tumor Microenvironment Following Exposure to SM-88 (Day 34)

Figure 3: **CD4, CD8 (Total Number) and CD4/CD8 Ratio**

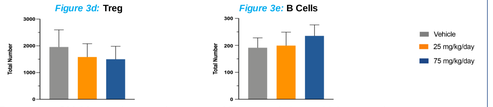
Figure 3a: **CD4** *Figure 3b:* **CD8** *Figure 3c:* **CD4/CD8 Ratio**



- To evaluate potential effects of SM-88 on the tumor immune microenvironment, flow cytometry was used to characterize the immune populations present in five randomly selected Pan02 tumors collected at study termination (Day 34).
- Exposure to SM-88 appears to decrease intra-tumoral CD4+ T cell populations, while preserving CD8+ populations, leading to decreases in the CD4+/CD8+ ratio.
- CD4/CD8 ratio was p= 0.015 for 75 mg/kg/day vs. Vehicle (ANOVA with Tukey post hoc).
- N = 5 for all treatment groups.

Figure 3: **Treg and B Cells**

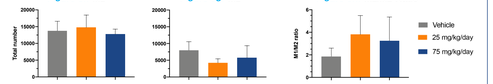
Figure 3d: **Treg** *Figure 3e:* **B Cells**



- Exposure to SM-88 may decrease intra-tumoral Treg populations, which may reduce immunosuppressive signaling within the tumor.
- Small increases in intracellular B cell populations were also observed following treatment with SM-88.
- N = 5 for all treatment groups.

Figure 3: **Macrophages (Total Number) and M1/M2 Ratio**

Figure 3f: **M1** *Figure 3g:* **M2** *Figure 3h:* **M1/M2 Ratio**



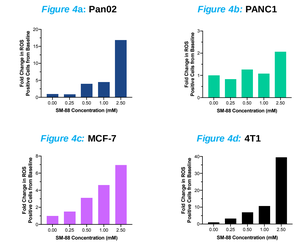
- Exposure to SM-88 may preserve M1 polarized macrophage populations while decreasing populations of M2 polarized macrophages.
- Within the tumor microenvironment, M1 macrophages have anti-tumor effects, while M2 macrophages often promote tumor growth.
- Thus, alterations in the M1/M2 ratio could contribute to suppression or reversal of tumor growth.
- N = 5 for all treatment groups.

SM-88 Induces Reactive Oxygen Species (ROS) Production

Figure 4: **ROS Positive Cells**

Figure 4a: **Pan02** *Figure 4b:* **PANC1**

Figure 4c: **MCF-7** *Figure 4d:* **4T1**



- The potential of SM-88 to increase ROS production was investigated using four cell lines, two pancreatic cancer (Pan02 and PANC1) and two breast cancer (4T1 and MCF-7).
- Dose dependent increases were observed in ROS production following 24 hours of exposure to SM-88 ME.

SM-88 Affects Autophagy

Figure 5a

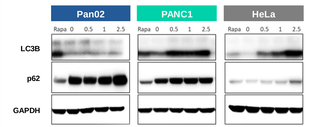
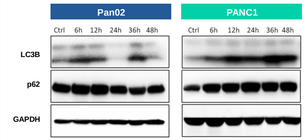


Figure 5b



- The potential of SM-88 to affect autophagy was investigated using three cell lines, two pancreatic cancer (Pan02 and PANC1) and one ovarian cancer (HeLa).
- Exposure to SM-88 ME appeared to promote a dose dependent increase in LC3B expression at 24 hours in PANC1 and HeLa cells (Figure 5a).
- Exposure to 1 mM SM-88 ME promoted an increase in LC3B expression beginning at least 6 hours after treatment in both Pan02 and PANC1 cells.
- SM-88 ME exposure either did not affect or slightly increased p62 expression.
- These data suggest that SM-88 affects autophagic mechanisms, and follow-up experiments are underway to determine whether the increases in LC3B expression are due to autophagy induction or blockade of autophagosome and lysosome fusion.

CONCLUSIONS

- The importance of amino acid metabolism in cancer has gained greater awareness over the past decade; however, potential therapeutic approaches in this area remain limited.
- SM-88 has been dosed in over 180 cancer patients and has shown encouraging efficacy and safety findings to date.
- Early data indicate that SM-88 may promote increases in ROS generation and alterations in autophagy in certain cancer cell types (Pan02 pancreatic cancer).
- Potential immune modulatory effects of SM-88, including alterations in macrophage polarization and other key tumor immune cells (CD4+ and Treg) are being examined.

DISCUSSION AND FUTURE DIRECTIONS

- SM-88 has demonstrated encouraging efficacy in 15 different tumor types. This may be due to the effects of SM-88 on cancer through multiple mechanisms, including immune modulation, alterations in autophagy and ROS induction.
- Alterations in autophagy have been associated with increased MHC1 expression in pancreatic cancer (Yamamoto et al., 2020). We are deepening our examination of the impact of SM-88 on autophagy/mitophagy, as well as any phenotypical changes that might be related to these changes including MHC1 expression.
- We are continuing to explore the immune modulatory effects observed in these preliminary experiments.
- Based on these and other ongoing experiments, we are beginning to explore SM-88 combinations with chemotherapies, targeted agents, or immuno-oncology therapies.
- We are continuing to explore these and other effects of SM-88, as well as potential biomarkers of SM-88 sensitivity, through experiments on human organoids, and metabolomic and gene expression analyses.

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