In Vivo Mouse Model Data Demonstrating Reduction in Tumor Cell Proliferation Following Intra-tumoral Administration of TYME-18

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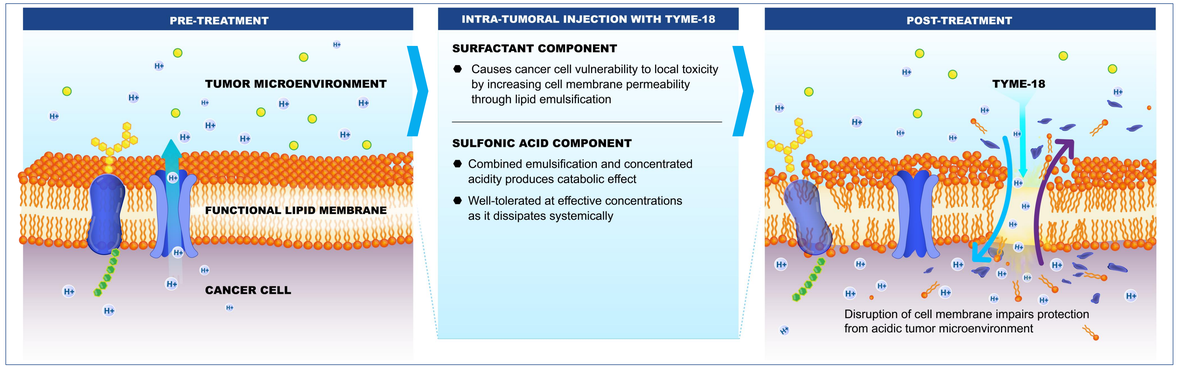
INTRODUCTION

- **TYME-18** is a combination of two agents administered intra-tumorally to induce tumor regression without injuring adjacent tissue.
  - ***Surfactant Component (proprietary combination solvent system):*** cause cancer vulnerability to local toxicity by increasing cell membrane permeability.
  - ***Sulfonic Acid Component:*** naturally occurring acid that is cytotoxic at high concentrations within the tumor but is readily broken down in the bloodstream when released systemically; sulfonic acids are lipid emulsifiers, and thus can disrupt cell membranes, especially in the presence of the penetration enhancers used here.
- The sulfonic acid component is approved for human use in dermatological procedures where it is injected into fatty regions to break down and emulsify adipose tissue.
- TYME-18 combines a novel repurposing of known agents with a proprietary solvent system to have utility in cancer-relevant and other clinical contexts (Thunangtong 2010; Di Ciaula 2017).
- At high concentrations, the sulfonic acid used in TYME-18 has lytic properties, and TYME's proprietary solvent system is designed to enhance penetration of the sulfonic acid into the tumor. Reducing the integrity of the cancer cell could disrupt its normal function, promoting cell rupture and exposure to the toxic tumor microenvironment.
- In many non-metastatic cancers, resection is the primary curative treatment. However, many patients possess lesions located in traditionally challenging locations (i.e., head/neck, pancreas), or those impinging on vital structures that make resection or radiotherapy, difficult or ill-advised.
- A safe and effective intra-tumoral therapy would have utility for treating unresectable or inoperable primary or metastatic tumors or where standard radiotherapy options may be considered higher risk.
- This intra-tumoral injection is designed to selectively kill cancer through modulating microenvironment toxicity while maintaining safety for healthy tissues, as displayed in the hypothesized MOA below:



PRELIMINARY DEVELOPMENT AND DOSE OPTIMIZATION

To date, several preliminary studies have been conducted to explore the potential anti-cancer effects of TYME-18.

PRELIMINARY PROOF-OF-CONCEPT STUDY:

- A preliminary proof-of-concept study was performed to evaluate the antitumor effect of TYME-18 in BALB/c mice bearing CT26 tumors (data not shown).
- Treatment with TYME-18 significantly inhibited CT26 tumor growth in BALB/c mice, compared with control (saline) ($p<0.01$, t-test of tumor volumes at treatment Day 13).
- 4 of 7 (57.1%) mice in the control group reached the pre-specified maximum allowed tumor volume, compared with 1 of 8 (12.5%) in the TYME-18 treated group (OR=0.11).
- 25% (2 of 8) mice treated with TYME-18 were tumor-free at the end of study, compared with 0% (0 of 7) in the control group.
- Initial histopathological examination of excised tumors revealed that animals treated with TYME-18 had notable tumor shrinkage and necrosis, compared with none of the animals in the control group.

DOSE OPTIMIZATION STUDY:

- In an initial dose optimization study, various doses and administration frequencies of TYME-18 were tested (data not shown).

RESULTS

Anti-tumor Effects of TYME-18

Figure 1

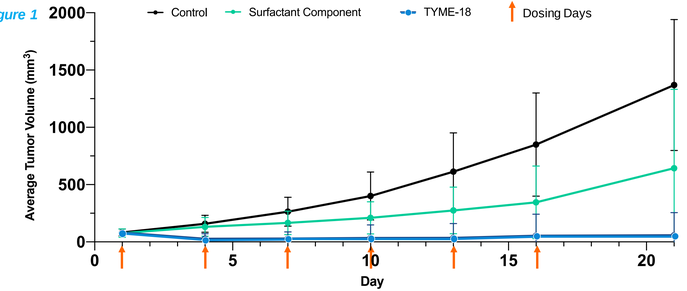


Table 1: AVERAGE TUMOR VOLUME (mm^3) BY DOSING/MEASUREMENT DAY

	1	4	7	10	13	16	21
Control	83.1	157.9	263.4	401.4	613.9	849.4	1368.9
Surfactant Component	76.4	131.1	166.3	209.6	274.3	345.7	642.8
TYME-18	80.5	28.2	28.9	34.2	35.8	54.4	57.2

Table 2: TUMOR-FREE MICE AT STUDY END (FISHER'S EXACT TEST $p < 0.0001$)

TREATMENT	# OF MICE	# TUMOR FREE MICE	% TUMOR FREE MICE
Control	20	0	0
Surfactant Component	12	1	8.33
TYME-18	12	11	91.6

- To investigate the effects of the individual components of TYME-18 on tumor growth, an *in vivo* xenograft experiment was conducted using the mouse colon cell line CT26 (Figure 1; Tables 1 and 2).
- A minor reduction in tumor cell mass was observed in response to the surfactant component, with the greatest response observed in the group treated with TYME-18 (surfactant component+sulfonic acid component) (ANOVA, $p=0.001$) (Figure 1; Table 1).
- 11 out of 12 (91.6%) mice treated with TYME-18 were tumor-free by study end (Table 2).
- Neither the control nor the surfactant component on its own produced similar responses to TYME-18 full combination.
- Results from this study replicate and confirm results observed in preliminary TYME-18 studies.

METHODS

COMPONENT EFFECT STUDY:

- Mice were implanted subcutaneously with CT26 cells. Tumors were allowed to grow for approximately six days before treatment initiation.
- Mice received intra-tumoral injections of TYME-18, or the surfactant component alone, or control.
- Six intra-tumoral injections were administered at 3-day intervals.
- Measurements were taken every 3 days. Animals were sacrificed when tumors reached 2000 mm^3 or at the end of the study.

CONCLUSIONS

- TYME-18 has been studied in three preclinical mouse xenograph studies, each with encouraging efficacy.
- The current studies were aimed to establish proof of concept, optimize dosing and treatment schedule, and confirm findings.
- In the component effect study, TYME-18 treatment resulted in 11/12 (91.6%) of established tumors resolving completely, compared with ~16x mean growth in the control treatment.
- While not a primary focus, no local or systemic toxicities were reported in treated mice.
- TYME-18 has demonstrated encouraging initial efficacy and warrants additional study.

DISCUSSION

- Local administration of TYME-18 led to significant tumor regression compared to rapid growth observed in control treatments.
- Sulfonic acids (similar to the one used in TYME-18) are recognized as important regulators of energy metabolism. Through effects on FXR, LXR, PPAR-α, and PPAR-γ, sulfonic acids can regulate important changes in energy metabolism including insulin utilization, insulin receptor sensitivity, and the transcription of the genes that mediate glucose and lipid metabolism, as well as various immune effects; all of which may contribute to potential anti-cancer effects (Di Ciaula 2017).
- Unfortunately, many cancer patients present with unresectable masses that could benefit from a well-tolerated, localized treatment. Additional treatment approaches are needed to optimally address these cases, either as standalone treatment, or as an adjunct to other local therapies, such as stereotactic radiotherapy, ablative or other such treatments.
- Further studies may better define the optimal dosing frequency, route and concentration for specific clinical uses.
- Currently, TYME-18 is under investigation as a simple and non-toxic treatment for tumors for which systemic therapy is not indicated.

REFERENCES

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