

# The HDAC Inhibitor Romidepsin Increases Survival in Mice with Lung Metastases from Highly-Metastatic Cell Lines that Phenocopy Patient-derived Sarcospheres

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**INTRODUCTION:** Osteosarcoma predominately affects adolescents and young adults. The five-year survival rate of those with detectable pulmonary metastases is only 30%. It is therefore necessary to identify novel therapies that target the progression of pulmonary metastases. Our lab previously screened 114 FDA-approved anti-cancer drugs to identify agents that decrease the growth of 3D spheroids (sarcospheres) generated from highly-metastatic osteosarcoma cell lines. Sarcospheres more closely mimic pulmonary metastases compared with a cell monolayer. The top hits included both histone deacetylase inhibitors (HDIs) that were tested [1]. In follow-up experiments measuring potency on sarcospheres and toxicity on non-transformed cells, romidepsin was the top hit of the 114-drug panel as well of the two additional FDA-approved HDIs and seven HDIs that are in clinical oncology trials. Our goal was therefore to further evaluate romidepsin as a potential therapy for metastatic osteosarcoma.

**METHODS:** Uniform sarcospheres (~400µm in diameter) were generated from established highly-metastatic human osteosarcoma cell lines and low-passage primary cells from canine and human osteosarcoma by our previously validated centrifugation-based method [2] and incubated with or without romidepsin for 48 hours. Viability was measured using resazurin reduction. Sarcosphere size and cytotoxicity were measured using the Incucyte® S3 Live-Cell Analysis System. *In vitro* data show mean ± SD of n=1-4 independent experiments with 6 sarcospheres at each dose. Cell cycle was analyzed by flow cytometry. To treat lung metastases directly, cells were implanted by tail vein injection and metastases were allowed to develop for 7 days before treatment with romidepsin (2.25 mg/kg, twice/week) or DMSO vehicle. When mice reached a humane endpoint, they were euthanized and lungs were fixed for histological analysis. One-way ANOVA with multiple comparisons was used to compare vehicle group with treated groups for both *in vitro* and *in vivo* experiments (\* p<0.05, \*\* p<0.003, \*\*\* p<0.001, \*\*\*\*p<0.0001).

**RESULTS:** Romidepsin potently decreased viability in sarcospheres from established highly-metastatic osteosarcoma cell lines (relative IC50s = 2-6nM, Fig 1A). ~50% of the canine and human (orange lines in Fig 1B-C) patient-derived sarcospheres had similar responses to romidepsin (relative IC50s = 0.05-26nM) suggesting that those patients might be good candidates for romidepsin therapy. In the absence of drug, viability doubled in 143B and MG63.3 sarcospheres during two days of culture (black bars in Fig 1D). In contrast, LM7 and K7M2 sarcospheres, and the majority of patient-derived sarcospheres show little change in viability or size in the absence of romidepsin (red bars in Fig 1D, black lines in Fig 2A-B). To increase translatability, mechanistic studies therefore focused on LM7 and K7M2 sarcospheres. Although romidepsin did not alter sarcosphere size, it induced cytotoxicity and increased the proportion of sub-G1 cells (Fig 2D-E) with potency similar to the effects on viability (Fig 1A). Most importantly, romidepsin reduced the area and number of lung metastases, and prolonged survival in mice with metastases of the K7M2 cell line (n=4, Fig 2F and not shown). In contrast, the increase in viability and size of 143B and MG63.3 sarcospheres during two days of culture (black bars in Fig 1D) is blocked by romidepsin due to a G2/M cell cycle arrest with no detectable increase in cytotoxicity (not shown and Fig 2D) and romidepsin had little to no efficacy on mice with lung metastases of the 143B cell line (n=10, not shown).

**DISCUSSION:** Romidepsin potently inhibited viability in ~50% of patient-derived sarcospheres including patients who had been heavily-pretreated with standard-of-care osteosarcoma chemotherapeutics. Sarcospheres from low-passage patient cells were phenocopied by sarcospheres from highly-metastatic LM7 and K7M2 osteosarcoma cell lines where romidepsin potently inhibited sarcosphere viability, induced cytotoxicity, and increased the proportion of sub-G1 cells. Most importantly, romidepsin prolonged survival in mice with lung metastases from the K7M2 cell line.

**CLINICAL RELEVANCE:** Romidepsin will be evaluated in mice with metastases from patient-derived cells and if effective, could be repurposed for canine and human clinical trials to improve outcomes in metastatic osteosarcoma without the associated costs and extensive timeframe of traditional drug discovery. Measuring romidepsin responses by patient-derived sarcospheres might be useful to identify patients likely to respond to romidepsin.

**REFERENCES:** [1] Collier, CD et al. 2016, *ORS Abstracts*, #0384; [2] Collier, CD et al. 2018, *CORR*, 476(7): 1400-1411

