HDAC1-specific inhibition upregulates *Malat1* in myeloid-derived suppressor cells and breast cancer cells and induces cell cycle arrest

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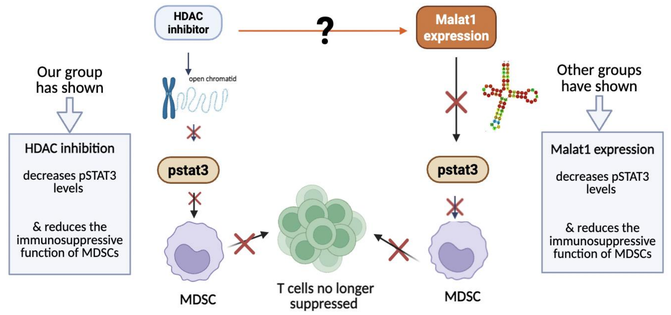
INTRODUCTION

- Immunotherapy is a novel cancer treatment that re-trains the body's immune system to fight cancer.
- Metastatic breast cancer tumors are resistant to these therapies due to myeloid-derived suppressor cells (MDSCs), which suppress T cells.
- Our group has reported that Class 1 histone deacetylase inhibition (HDACi) with epigenetic modulator Entinostat reduces MDSC immunosuppressive function.
- However, the molecular mechanism remains unclear.



- There may exist a mechanistic link between HDACi and lncRNA *Malat1.*
- HDACi has been shown to affect cell cycle progression in cancer cells.

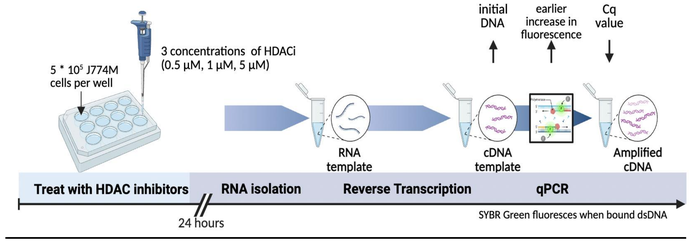
Hypothesis: Class I HDAC inhibition will upregulate *Malat1* and induce cell cycle arrest in MDSCs.

METHODS

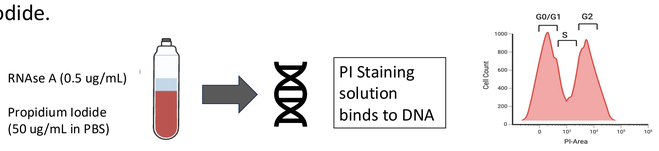
MDSC-like J774M cells and murine (4T1, NT2.5,NT2.5-LM, NT2.5-LV) and human (MCF-7, MDA-MB-231) breast cancer cell lines were treated with a panel of 12 HDAC inhibitors.

	ENT	PAN	BEL	VOR	TSA	DOM	CHL	PYR	SCA	RGF	TMP	SIS
Class	I	I/II/IV	I/II/IV	I/II/IV	I/II	I	I	I	I	I	IIA	IV
HDACs	1/2/3	pan-	pan-	pan-	pan-	1/2/3	1	1	2	3	4/5/7/9	11
FDA status		Refractory MM 2015	T-cell lymphoma 2014	CTCL 2006	ENT: Entinostat PAN: Panobinostat BEL: Belinostat		VOR: Vorinostat TSA: Trichostatin A DOM: Domatinostat		CHL: Chlopynostat PYR: Pyroxamide SCA: Santa Cruzamate A		RGF: RGFP966 TMP: TMP269 SIS: SIS17	

SYBR green master mix-based qPCRs were performed to measure *Malat1* expression relative to housekeeping gene, β-actin in murine cell lines and GAPDH in human cell lines.



Cell cycle distribution was determined by flow cytometry analysis of HDACi treated, 70% EtOH fixed J774M cells stained with propidium iodide.



HDAC1 inhibition increases *Malat1* expression in MDSCs

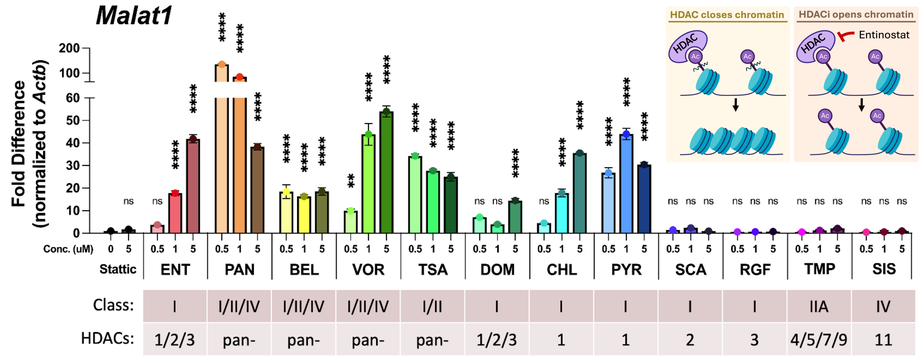


Fig. 1: Increased lncRNA *Malat1* expression in MDSCS is HDAC1 dependent

Malat1 expression in J774M cells treated with 12 HDAC inhibitors at 3 concentrations (0.5, 1, and 5 µM) relative to vehicle control DMSO treatment. ns = not significant * p<0.05, *** p< 0.001, **** p<0.0001.

HDAC1 and HDAC3 inhibition induces G0/G1 cell cycle arrest in MDSCs

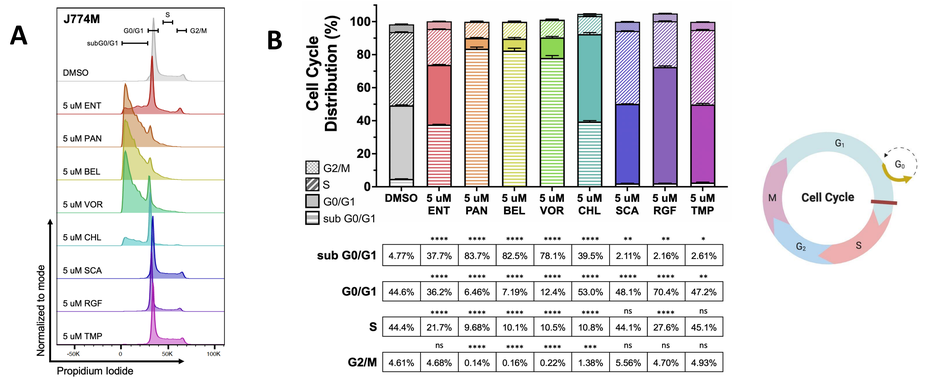


Fig. 2: G0/G1 cell cycle arrest in MDSCs is HDAC1 and HDAC3 dependent

A. Flow cytometry plot reveals shift in propidium iodide fluorescence and **B.** quantifications of cell cycle phase distributions in J774M cells after 24 hours of HDAC inhibitor treatment. ns = not significant, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

HDAC1 inhibition increases *Malat1* expression in breast tumor cell lines

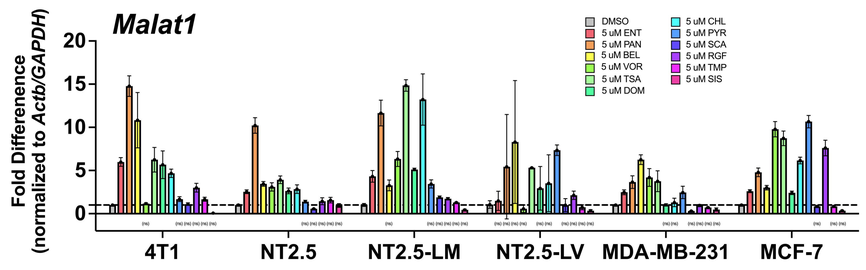


Fig. 3: Increased lncRNA Malat1 expression in murine and human breast cancer cell lines is HDAC1 dependent

Malat1 expression in murine (4T1, NT2.5, NT2.5-LM, NT2.5-LV) and human (MCF-7, MDA-MB-231) breast cancer cell lines treated with 12 HDAC inhibitors at a concentration of 5 µM relative to vehicle control DMSO treatment. ns = not significant.

Pan-HDAC inhibitors Belinostat and Vorinostat are non-inferior to Entinostat + dual ICI in NT2.5 mouse model

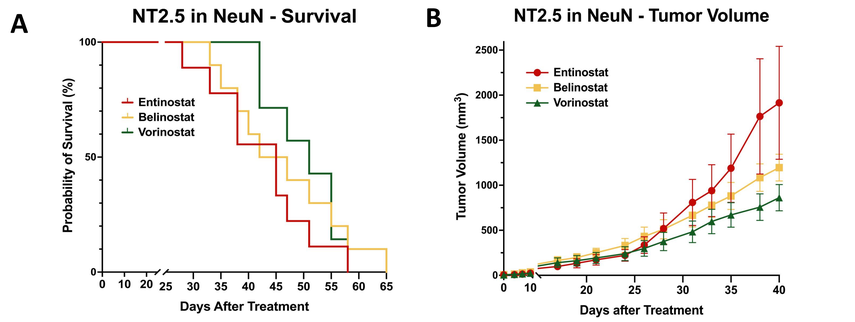
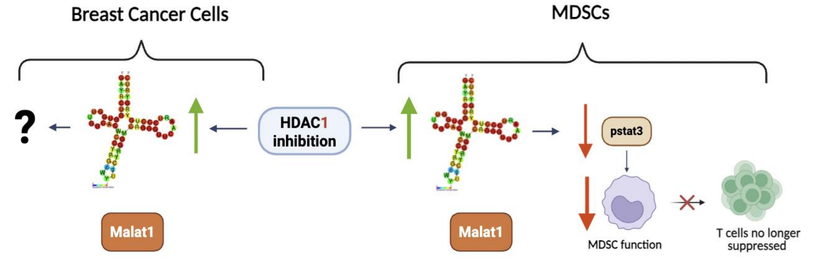


Fig. 4: Treatment with pan-HDACis are non-inferior to treatment with Class I HDACi Entinostat in a syngeneic model of breast cancer

A. Kaplan–Meier survival curve of the NT2.5 syngeneic mouse model treated with HDACi in combination with dual ICI (anti–PD-1 + anti–CTLA-4). Vorinostat shows a trend toward improved survival. However, this was not statistically significant. **B.** Tumor growth curves demonstrate that tumor volume was not significantly different across HDACi treatment.

CONCLUSIONS

- Our findings identify HDAC1 as a regulator of *Malat1* expression in MDSCs.
- This suggests a *Malat1*-mediated mechanism of HDAC1 inhibition to induce cell cycle arrest and potentially reduce MDSC suppression of T cells.
- We also identify HDAC1 as a regulator of *Malat1* expression in breast cancer cells, adding to the literature of *Malat1's* conflicting role as a context-dependent pro-proliferative vs. metastasis-suppressing lncRNA.



HDACi affects myeloid suppression to improve response to ICIs in preclinical studies and Phase I studies (NCI-9844) in patients. Understanding the mechanism of altered suppression by HDACi could broaden the use of this promising treatment in patients with metastatic breast cancer.

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