11P - Novel HDAC6 inhibitors show anti-lymphoma activity alone and in combination with venetoclax and copanlisib

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Background

Diffuse large B cell lymphomas (DLBCL) are aggressive tumours with frequent aberrations in epigenetic proteins. Histone deacetylase inhibitors (HDACi) are epigenetic agents with pre-clinical and clinical efficacy in lymphomas. Here we investigated the *in vitro* anti-lymphoma activities of eight novel HDAC6 inhibitors (HDAC6i) in DLBCL.

Methods

MTT assay was used to assess the anti-proliferative activities of HDAC6i: SS-2-08, SS-5-55, SW-101, SW-113, SW-114, SW-115, SW-117 and WT-36-87, alone or combined with venetoclax or copanlisib (72 hours [hrs]) in DLBCL cells: DOHH2, OCI-LY-18 (*BCL2* and *MYC* translocated); SU-DHL-4, OCI-LY-1 (BCL2 translocated and MYC amplified). The Chou-Talalay combination index (CI) determined additive effect (CI 0.9-1.1), synergism (CI < 0.9) and antagonism (CI > 1.1). For cell cycle analysis by flow cytometry, cells were fixed in 70% ethanol then stained with 7-AAD. Western blotting determined levels of acetylated α-tubulin.

Results

Figure 1

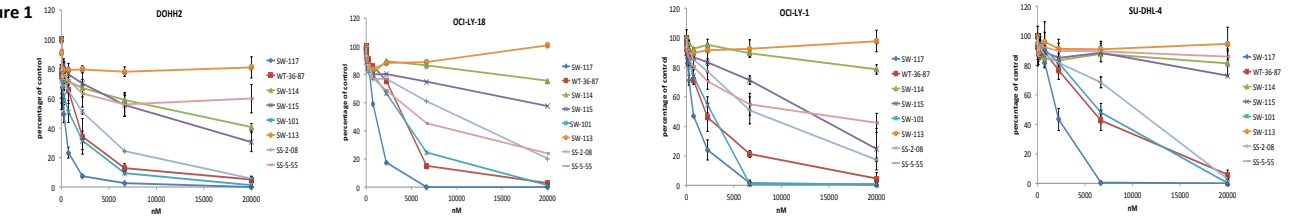

Figure 2

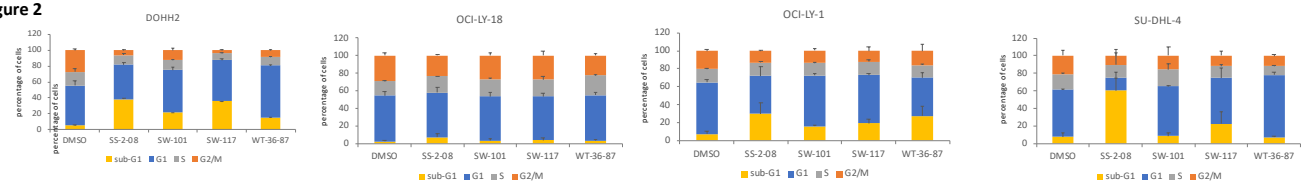

Figure 3

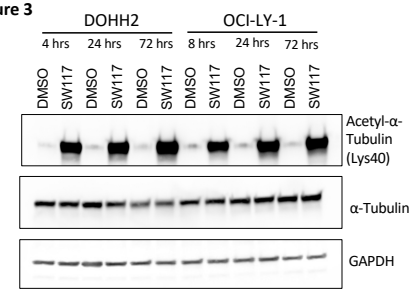

Figure 1. Novel HDAC6 inhibitors inhibit the proliferation of DLBCL cells. Each graph shows the dose response curves for a DLBCL cell line treated with increasing concentrations of each of the eight HDAC6i for 72 hrs.

Figure 2. DLBCL cells were treated with the median IC50 concentration of each of the four most potent HDAC6i for 72 hrs. The HDAC6i induced G1 arrest and cell death, as indicated by accumulation of cells in sub-G1.

Figure 3. DOHH2 and OCI-LY-1 cells were treated with the most potent HDAC6i, SW-117, for 4, 24 and 72 hours. SW-117 strongly upregulated acetylated α-tubulin already after 4 hours, with the strongest upregulation seen after 72 hrs.

Table 1. IC50 values (μM) obtained for DLBCL cells treated with each of the eight HDAC6i. >20 indicates that the IC50 was higher than the maximum concentration used for the MTT assay (20 μM).

Tables 2 and 3. Combination indexes (CI) obtained when the four most potent HDAC6i were combined respectively, with venetoclax or copanlisib. Synergism: CI < 0.9, additive: CI 0.9 -1.1, antagonism: CI > 1.1; 95% C.I., 95% confidence interval; N/A, not applicable.

Table 1

Cell line	SS-2-08	SS-5-55	SW-101	SW-113	SW-114	SW-115	SW-117	WT-36-87
DOHH2	1.2	>20	0.6	>20	7.8	10.6	0.2	1.9
SU-DHL-4	8.3	>20	6.2	>20	>20	>20	2.0	5.6
OCI-LY-18	12.6	18.3	1.9	>20	>20	>20	0.8	3.2
OCI-LY-1	7.1	20.3	2.2	>20	>20	10.1	0.5	1.5
MEDIAN IC50	7.7	>20	2.0	>20	>20	>20	0.6	2.6

Table 2

Venetoclax	DOHH2	95% C.I.	SU-DHL-4	95% C.I.
SW-117	0.2	0.2 - 0.3	1.2	1.0 - 1.3
SW-101	0.6	0.5 - 0.8	1.6	1.5 - 1.8
WT-36-87	0.1	0.1 - 0.1	>3	N/A
SW-113	>3	N/A	1.0	1.0 - 1.1

Table 3

Copanlisib	DOHH2	95% C.I.	SU-DHL-4	95% C.I.
SW-117	0.8	0.7 - 0.9	1.0	0.7 - 1.1
SW-101	0.7	0.6 - 0.8	1.2	0.9 - 1.5
WT-36-87	1.2	1.1 - 1.4	>3	N/A
SW-113	>3	N/A	1.3	1.1 - 1.4

Median IC50 values for the eight HDAC6i ranged from 0.6 μM to 19.3 μM. SW-101, SW-117, SS-2-08 and WT-36-87 showed the strongest anti-proliferative activities. SW-117 was the most potent (range 0.2 - 2 μM; median 0.6 μM). DLBCLs treated with median IC50s of SW-101, SW-117, SS-2-08 and WT-36-87 for 72 hrs underwent G1 arrest and cell death. SW-117, with a potency of 0.3 nM against HDAC6, increased acetylated α-tubulin levels at 4 hrs and this upregulation persisted to 72 hrs.

The three most active HDAC6i (SW-117, SW-101, WT-36-87), plus SW-113 that showed negligible activity as a single agent, were tested in combination with the PI3Kα/δ inhibitor copanlisib and the BCL2 inhibitor venetoclax, in DOHH2 and SU-DHL-4. All four HDAC6i showed enhanced anti-proliferative activity in at least one of the combinations tested. SW-117 and SW-101 showed similar benefit when combined with either copanlisib or venetoclax.

Conclusions

We observed robust *in vitro* anti-lymphoma activity of novel HDAC6i in DLBCL cells. Our results suggest that these agents are worthy of further pre-clinical investigation in DLBCL as single agents and in combination with other targeted anti-lymphoma drugs.

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