



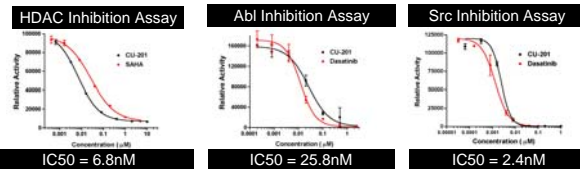
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Introduction

Single or multi-targeted agents that inhibit tyrosine kinases (TKI) are considered promising therapeutics against cancer, but often have limited clinical utility due to the heterogeneity of tumors and multiple mis-regulated pathways of tumor growth/survival. In addition, the emergence of drug resistance through mutation and activation of original or additional survival pathways help tumors evade TKI-mediated cell death. Agents inhibiting the non-kinase target, such as histone deacetylase inhibitors (HDAC) might have the potential to block survival pathways more broadly when used in combination with TKI. We have therefore designed a multi-targeted single small molecule, CU-201, that inhibits HDAC as well as Abl and Src family kinases, the combination being confirmed for potential synergistic interaction. CU-201 exhibits anti-proliferation activity against a broad range of cancer cell types, including cell lines that are insensitive to dasatinib and nilotinib. CU-201 could have clinical advantages of single-agent delivery, simultaneous inhibition of synergistic targets within tumors, and efficacy against various kinds of cancer.

1. CU-201 Potently Inhibits HDAC, Abl and Src Family Kinases



	HDAC	ABL	SRC	IC50 (nM)	Lck	c-Kit	PDGFRb
CU-201	6.8	25.8	2.4	2.4	1.2	2.4	4.9
Dasatinib	N/A	13.9	0.6	2	1.3	0.6	1.1

3. Rationale for CU-201 Design: Synergism Between Kinase & HDAC Inhibition

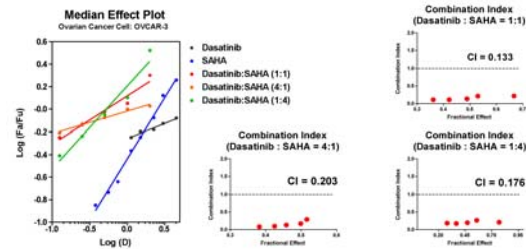
A simple method for the quantitative analysis of combined drug effects (Chou and Talalay, 1984)

Median-effect plot analysis determines whether drug combinations are synergistic

$$\text{Combination Index} = \frac{C1}{(EC50)1} + \frac{C2}{(EC50)2}$$

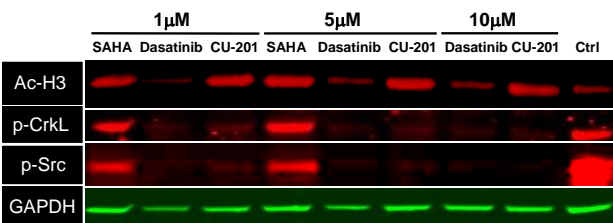
CI < 1, Synergistic
CI = 1, Additive
CI > 1, Antagonistic

SAHA and Dasatinib were used as reference compounds to inhibit HDAC and kinases, respectively



- Synergism was observed over a 16-fold range of ratios of combined HDAC & Bcr-Abl/Src inhibitory reference compounds, showing that specific ratios are not required
- Potential for more effective treatment of heterogeneous and drug-resistant tumors

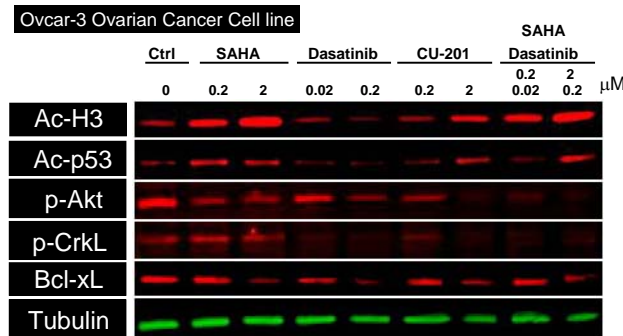
2. CU-201 Inhibits HDAC, Bcr-Abl and Src Pathways in Tumor Cells



Western blot analysis

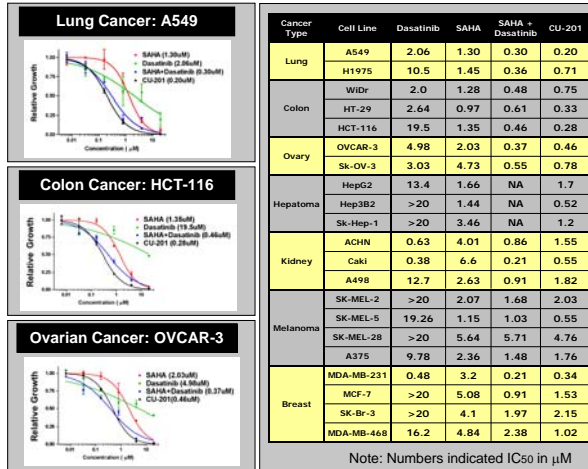
- CU-201 causes the accumulation of acetylated Histone-H3, confirming HDAC inhibition activity of CU-201 in cultured cancer cells
- CU-201 inhibits the phosphorylation of CrkL, a key regulator of Bcr-Abl signaling in cancer cells
- CU-201 inhibits phosphorylation of Src and Lyn of Src family kinases.

4. Potential Mechanism for Synergism: CU-201 Synergistically Inhibits Akt Signaling

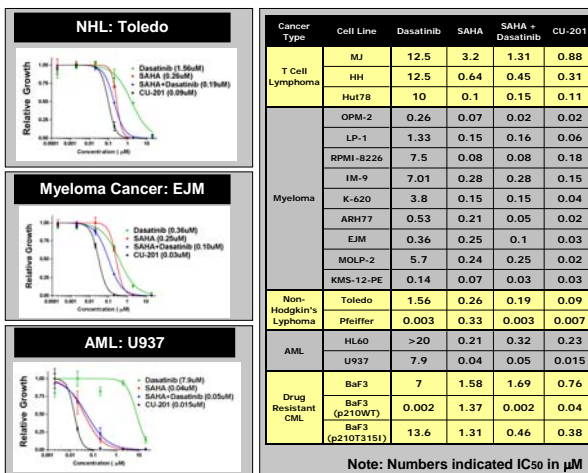


- Inhibiting HDAC (w/ SAHA) and kinase signaling (w/ dasatinib) together synergistically induced the depletion of activated Akt (p-Akt↓)
- CU-201 alone also synergistically induced the depletion of activated Akt
- The demonstration of increased acetylated Histone-H3 and p53 further confirms that CU-201 inhibits HDAC activity in cultured cancer cells
- CU-201 also inhibits the phosphorylation of CrkL, a key regulator of Bcr-Abl signaling

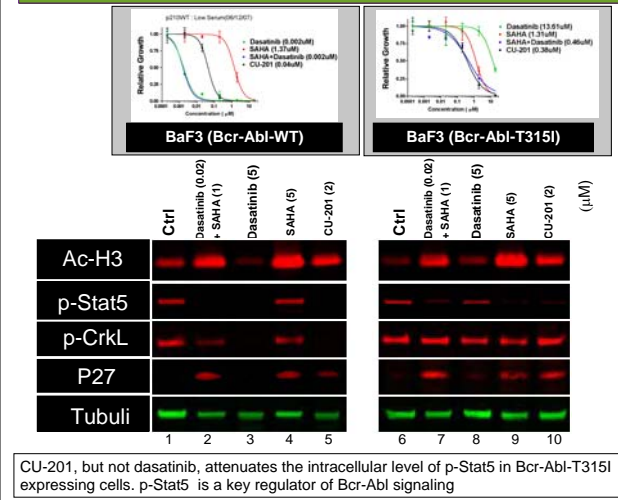
5. CU-201 Effectively Inhibits Proliferation of Human Solid-Tumor Derived Cancer Cell Lines



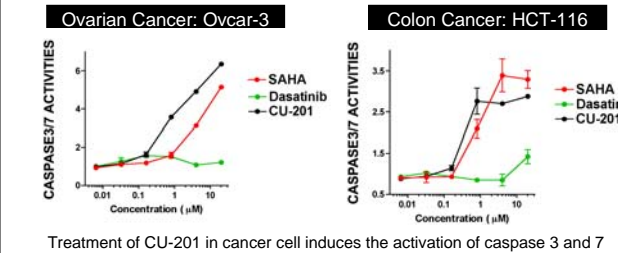
6. CU-201 Effectively Inhibits Proliferation of Human Hematological Cancer Cell Lines



7. CU-201 Displays Strong Potency in a Dasatinib-Resistant Cell Line of CML Model



8. CU-201 Induces Apoptosis in Cultured Cancer Cells



Conclusions

- CU-201, a potent HDAC, Bcr-Abl and Src family inhibitor, synergistically inactivates p-Akt or p-Stat5 pathways, which mechanistically should improve kinase inhibitory responses
- CU-201 displays greater anti-proliferation and apoptosis-inducing potency against a broad range of cultured cancer cell lines than approved Bcr-Abl/Src or HDAC inhibitors
- These results suggest that CU-201 could overcome limitations observed in the treatment of heterogeneous and drug-resistant tumors by traditional kinase inhibitors
- CU-201, as a single agent with potent non-kinase and kinase inhibitory activities, might have the potential for efficacy and safety advantages over individual or combinations of approved targeted agents against a range of both hematologic and solid tumor cancers