

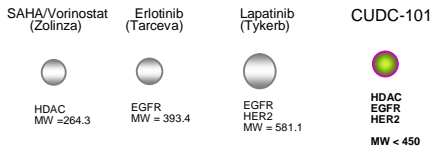
# Potent Anti-Cancer Activity *In Vitro* and *In Vivo* by a Novel, Small Molecule Inhibitor of HDAC, EGFR and Her2

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## Introduction

EGFR and Her2 are involved in the pathogenesis of many solid human cancers and have been validated clinically as targets for cancer therapy. Histone deacetylases (HDACs) are also clinically validated targets for cutaneous T-cell lymphoma and evidence of their involvement in solid tumors is accumulating. Several HDAC inhibitors are under development and one such inhibitor, SAHA (Merck), has received FDA approval. Potential synergy between EGFR and HDAC inhibition has been suggested by several publications and our in-house results. Curis is pursuing a multi-target approach using its proprietary Multi-target Drug Development Platform in order to develop small molecule compounds which inhibit multiple cancer targets simultaneously. Its leading compound CUDC-101 (MW <450), which targets HDAC as well as EGFR and Her2, displays anti-proliferation and apoptosis-inducing activities *in vitro* and *in vivo* against a broad range of cancer cell types.

**Figure 1. CUDC-101 Is Designed to Inhibit Three Clinically Validated Cancer Targets, HDAC, EGFR and Her2**



CUDC-101 is specifically designed to optimize the potency and mechanistic synergy of the individual targets. This confers unanticipated properties to the molecule (e.g., molecular weight, mechanistic advantages).

**Table 1. Synergistic Effects of HDAC and EGFR Inhibition in NSCLC and Breast Cancer Cell Lines**

Cell Line	Tumor Type	CI	Effect
MDA-MB-468	Breast	0.277	Synergy
MCF-7	Breast	0.63	Synergy
H2122	NSCLC	0.35	Synergy
H1703	NSCLC	0.68	Synergy

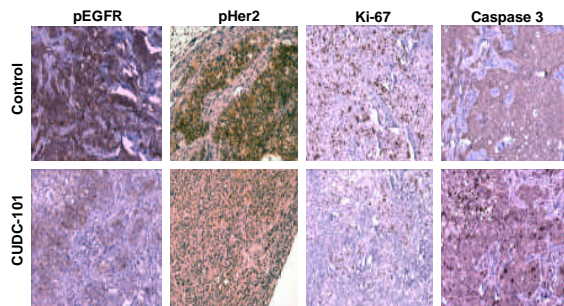
A method for the quantitative median-effect plot analysis of combined drug effects reported by Chou and Talalay was used to determine whether the tested drug combination, SAHA : erlotinib at a 1:1 ratio acts in an additive (calculated combination index or CI=1), synergistic (CI<1) or antagonistic manner (CI>1).

**Table 2. CUDC-101 is a Selective and Potent HDAC, EGFR and Her2-Inhibitor**

Compound	IC <sub>50</sub> (nM) in Enzyme Assays		
	HDAC	EGFR	HER2
Saha	40.0	NA	NA
Erlotinib	NA	48.0	134.5
Lapatinib	NA	TBD	10.2
<b>CUDC-101</b>	<b>4.4</b>	<b>2.4</b>	<b>15.7</b>

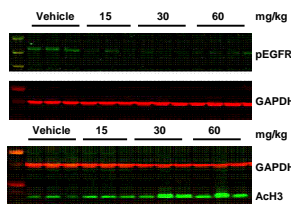
CUDC-101 inhibits the EGFR kinase, the Her2 (ErbB2) kinase and HDAC with high specificity. Its low nanomolar potency against EGFR is increased approx. 20-fold over a prototype EGFRi and 10-fold over a known HDACi. CUDC-101 displays similar potency to that observed with a known HER2i.

**Figure 2. CUDC-101 Inhibits EGFR and Her2 Phosphorylation, Decreases Cell Proliferation and Induces Apoptosis in HCC827 NSCLC Xenografts**



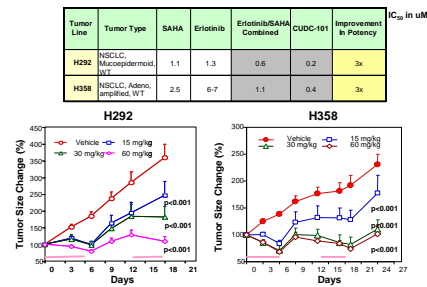
The pharmacodynamic effects of CUDC-101 were evaluated in human HCC827 xenografts grown in nude mice. In addition to a decrease of phosphorylation of its targets EGFR and HER2, the cell proliferation marker Ki67 is decreased after compound administration. Cleaved caspase 3, an early marker of apoptosis is induced, as evidenced by immunohistochemistry for this marker.

**Figure 3. CUDC-101 Inhibits EGFR and Induces Upregulation of Acetylated Histone H3 in a Dose-Dependent Manner**



Western blot analysis of xenograft tissue after CUDC-101 administration reveals that CUDC-101 inhibits EGFR phosphorylation and increases histone H3 acetylation in a dose-dependent manner, indicating *in vivo* activity on both targets. The observed molecular events contribute to the anti-tumor effect of CUDC-101 observed in the above-mentioned efficacy studies.

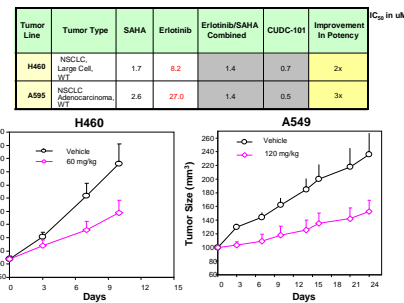
**Figure 4. CUDC-101 is Efficacious in EGFRi-Sensitive NSCLC Cell Lines and Xenografts**



For all xenograft studies, human tumor cells were grown subcutaneously in immunosuppressed mice. Briefly, athymic nude mice (CD-1 nu/nu) at age 6-8 weeks were obtained from Charles River laboratories and injected into the flank with single-cell suspensions of greater than 90% viability. Animals were randomized to different treatment groups and tumor size measured with a caliper. The following formula was used to calculate the tumor volume: Tumor volume = (length X width<sup>2</sup>)/2. Mice were monitored for general health and no drug-related toxicities or deaths were observed.

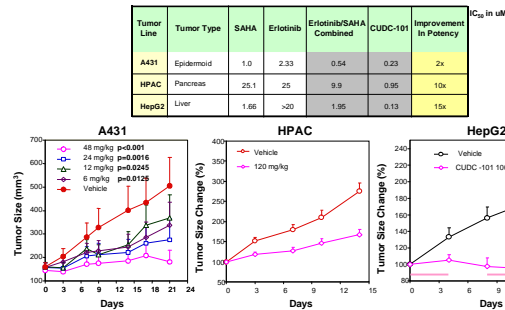
CUDC-101 induces tumor stasis/regression in EGFRi sensitive NSCLC cell lines. Pink bars denote days of compound administration.

**Figure 5. CUDC-101 is Efficacious in EGFRi-Insensitive NSCLC Cell Lines & Xenografts**



CUDC-101 inhibits tumor growth in EGFRi insensitive/resistant cell lines.

**Figure 6. CUDC-101 is Efficacious in Cell Lines and Xenografts of Other Cancer Types (Epidermoid, Pancreatic and Hepatocellular Cancer)**



CUDC-101 inhibits tumor growth in some cell xenografts and induces tumor stasis/regression in others.

## Conclusions

CUDC-101 is a very potent HDAC, EGFR and Her2 inhibitor which displays potency improvements over FDA-approved receptor tyrosine kinase (RTK) and HDAC inhibitors in multiple cell lines across various cancer types. In cancer xenograft models, CUDC-101 has been demonstrated to inhibit all three targets dose-dependently in a similar manner to *in vitro* studies. These target inhibition results in apoptotic cell death and inhibition of tumor cell proliferation. After treatment with CUDC-101, tumor regression has been observed in three tumor models which have been reported to be sensitive to RTK inhibitors. Tumor regression has also been noted in animal models of other tumor types (HepG2 etc.). Most importantly, anti-tumor effects of CUDC-101 have also been observed in animal tumor models reportedly insensitive or resistant to the marketed inhibitors. A favorable safety profile has also been observed in these preclinical studies as well as non-GLP toxicology studies in various species. These results support the notion that CUDC-101 is potentially suitable for future clinical development for various cancer indications.

## References

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