#4689

A Novel Tumor-specific HSP90 Inhibitor with Long Lasting Biological Activity

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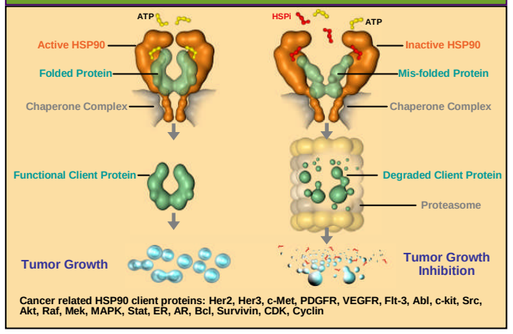
Introduction

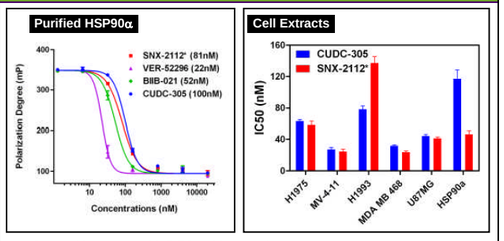
HSP90 is the core component of a multi-molecule chaperone complex formed by heat shock proteins and their close relatives. While a basal level of HSP90 facilitates normal protein folding and guards the proteome from mis-folding and aggregation, elevated expression of HSP90 has been observed in many tumor types. Increasing evidence indicates that HSP90 plays a major role in tumor cell survival and tumor progression by maintaining the aberrant activities of oncogenic kinases and signaling molecules. Inhibition of Hsp90 has been shown to lead to a decrease of these oncogenic proteins and has become a promising therapeutic strategy. Here we describe a novel synthetic small molecule HSP90 inhibitor, CUDC-305, as a drug candidate for the treatment of various types of cancers.

CUDC-305 Targets HSP90 to Blocking Its Chaperone Role for Cancer-associated Client Proteins



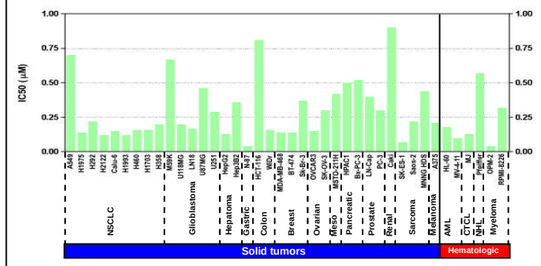
Cancer related HSP90 client proteins: Her2, Her3, c-Met, PDGFR, VEGFR, Flt-3, Abl, c-kit, Src, Akt, Raf, Mek, MAPK, Stat, ER, AR, Bcl, Survivin, CDK, Cyclin

CUDC-305 Binds HSP90 Proteins with High Affinity



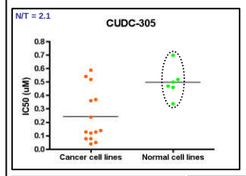
- Competition assay with FITC-geldanamycin
- Cells extracted with RIPA buffer
- SNX-2112 is the active metabolite of Serenex' clinical candidate SNX-5422, a pro-drug

CUDC-305 Is Highly Potent Against a Broad Range of Tumor Cell Lines

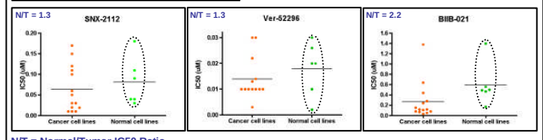


- 34 solid tumor cell lines & 6 hematologic cancer cell lines
- The median IC50 = 220 nM (range 40 to 900 nM)
- All IC_{50s} are well below concentrations achieved in tumor tissue (~4 to 20 uM)

CUDC-305 Displays Greater Specificity for Tumor Cells vs. Normal Cells and a Tighter IC50 Range in Normal Cells

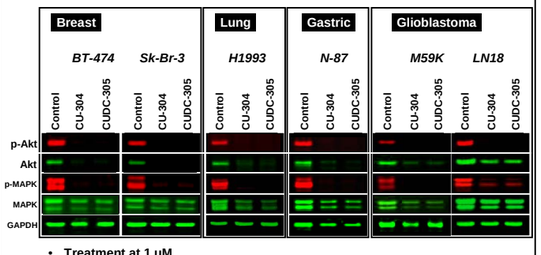


Normal Human Primary Cell Lines
- Dermal microvascular endothelial cells
- Hepatocytes
- Skeletal muscle cells
- Astrocytes
- Aortic smooth muscle cells
- Pulmonary artery smooth muscle cells



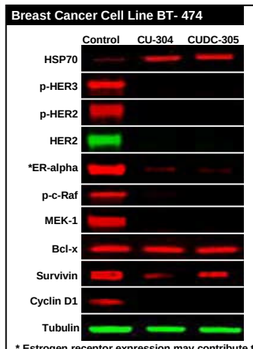
N/T = Normal/Tumor IC50 Ratio

CUDC-305 Suppresses Akt and MAPK Signaling In Different Cancer Cell Lines

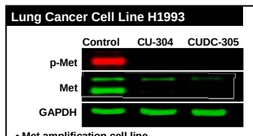


- Treatment at 1 uM
- CU-304 is an analogue of CUDC-305

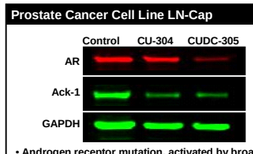
CUDC-305 Decreases the Level of Oncogenic HSP90 Client Proteins



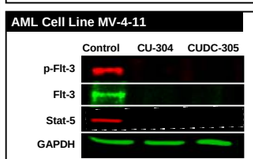
* Estrogen receptor expression may contribute to the escape of Her2 inhibition



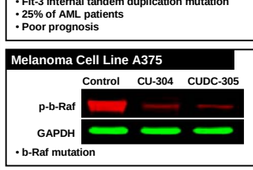
- Met amplification cell line
- Erlotinib-resistant



- Androgen receptor mutation, activated by broader range of steroids

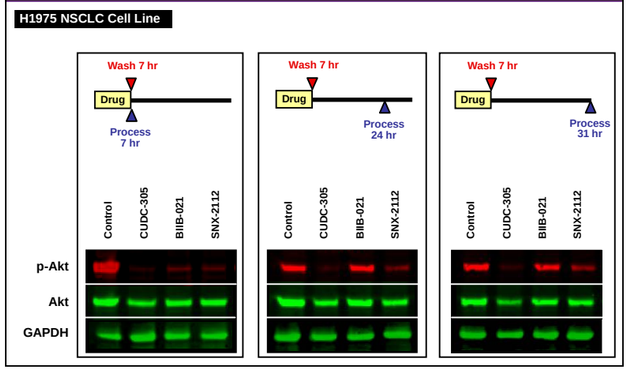


- Flt-3 internal tandem duplication mutation
- 25% of AML patients
- Poor prognosis

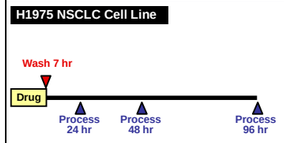


- b-Raf mutation

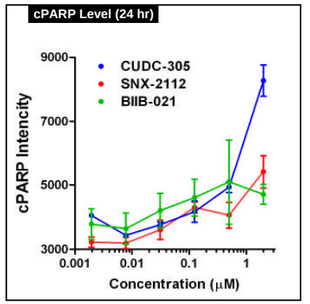
CUDC-305 Displays Prolonged Activities in Decreasing HSP90 Client Protein Levels

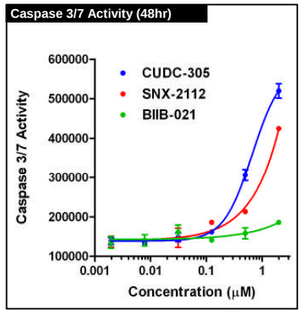


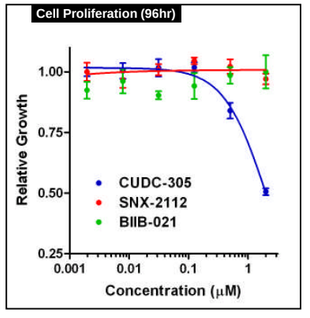
CUDC-305 Displays Prolonged Activities in Apoptosis Induction and Anti-proliferation



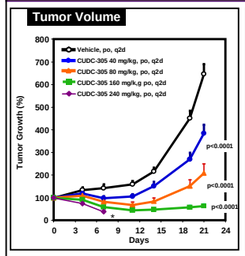
- cPARP level is detected by Cellomics High Content Imaging
- Caspase 3/7 activity is detected by fluorometric kit
- Cell proliferation is detected by ATP assay

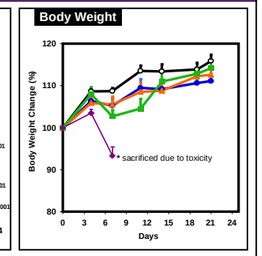


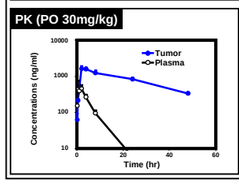




CUDC-305 Induces Regression in the U87MG Glioblastoma Subcutaneous Xenograft Model

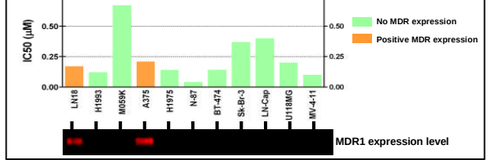






- Oral bioavailability is 96.0%
- High brain penetration
- Tumor half-life is 20.5 hrs with high Cmax & AUC
- Plasma half-life is 6.7 hrs
- Dose not induce significant weight loss
- No toxicity observed in animal studies

Potency of CUDC-305 Is Not Affected By Multiple Drug Resistant (MDR) Transporter Expression



Conclusions

Our results indicate that CUDC-305 efficiently decreases the levels of oncogenic HSP90 client proteins, induces apoptosis and inhibits cell proliferation against various cancer cell types. In addition, it displays a favorable safety profile *in vitro*, such as higher affinity to cancer chaperone complex, higher tolerance among different types of primary human cells, and prolonged biological activity. Consistently, CUDC-305 is well tolerated and induces tumor regression in xenograft models. Our data also indicate that the potency of CUDC-305 is not affected by the expression level of Multiple Drug Resistant transporter (MDR1), suggesting CUDC-305 is not subject to efflux transport by MDR1. In conclusion, our data indicate that CUDC-305 is a promising and potent drug candidate for cancer therapy.