Measurement of CUDC-101 Target Inhibition in Circulating Tumor Cells using a Fluorescent-Based, Quantitative Assay

Chris Neal, Jacky Woo, Sujita Sukumaran, and Darren W. Davis
ApoCell Inc., Houston TX 77054 USA

Background

The occurrence of receptor signaling redundancy in multiple cancer pathways has led to the growing development of more potent, multi-kinase inhibitors that have the capability to block a broad array of tumor-related activation pathways. CUDC-101, is a clinical stage, network targeted agent designed to simultaneously inhibit EGFR, HER2 and HDAC in tumor cells. The ability to monitor the various activities of CUDC-101 in readily assessable clinical samples may be informative for drug development processes including providing evidence of a pharmacodynamic effect in early clinical studies.

Objective

To develop quantitative laser scanning cytometry (LSC) assays for measuring total and phosphorylated EGFR and HER2, and acetylated histone H3 protein levels in circulating tumor cells (CTCs) to be used for pharmacodynamic monitoring of biomarkers of CUDC-101 target inhibition.

Materials & Methods

•Two head and neck cancer cell lines, SCC-9 and SCC-15, were treated with vehicle or CUDC-101 (10 µM, provided by Curis Inc, Lexington, MA, USA).

•CTCs (DAPI+CK+CD45-) from head and neck, or breast cancer patient blood samples treated *ex vivo* with CUDC-101 (10 µM) were enriched using the CellSearch® Profile Kit.

•The protein levels (mean fluorescence intensity, MFI) of pathway-specific molecules (total and phosphorylated EGFR and HER2, and acetylated histone H3) were quantified by laser scanning cytometry.

Results

Treatment of SCC-9 cells with CUDC-101 reduced EGFR and HER2 phosphorylation and increased the acetylation of histone H3

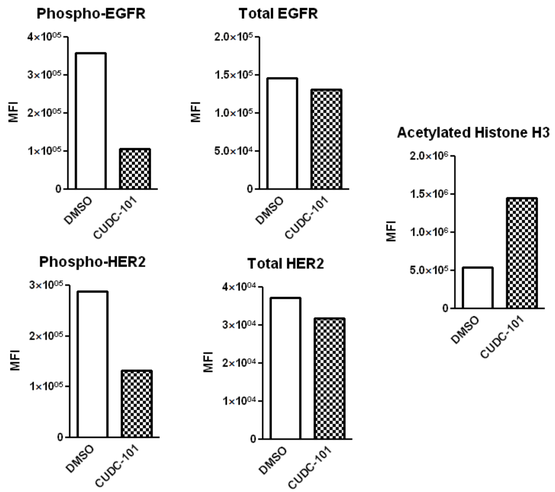

Figure 1. LSC analysis demonstrated that treatment of the SCC-9 cell line with CUDC-101(10 µM) led to a 70.1% reduction in pEGFR, and a 53.9% reduction in pHER2. Conversely, CUDC-101 treatment of SCC-9 cells led to a 165% increase in acetylated histone H3.

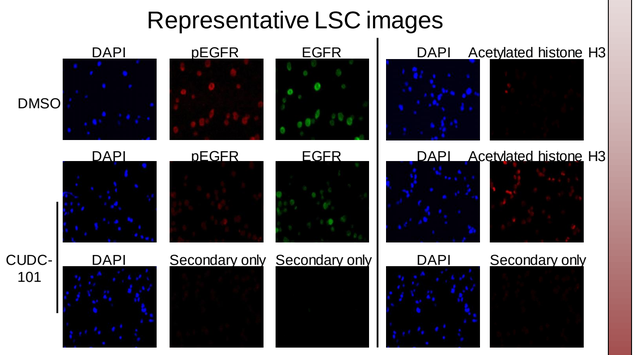

Figure 2. Representative LSC images demonstrated modulation of pEGFR, EGFR and acetylated histone H3 protein levels in SCC-9 cells treated with CUDC-101 .

Significant reduction of EGFR and HER2 phosphorylation in CTCs enriched from cancer patient blood treated *ex vivo* with CUDC-101

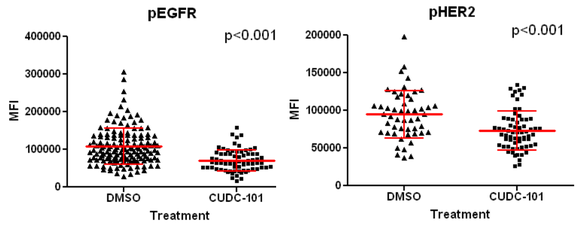

Figure 3. *Ex vivo* treatment of cancer patient blood with CUDC-101 compound resulted in a decrease in pEGFR and pHER2 expression in CTCs. Each dot represents one CTC.

CUDC-101 inhibited EGFR and HER2 phosphorylation in CTCs isolated from *ex vivo*-treated cancer patient blood

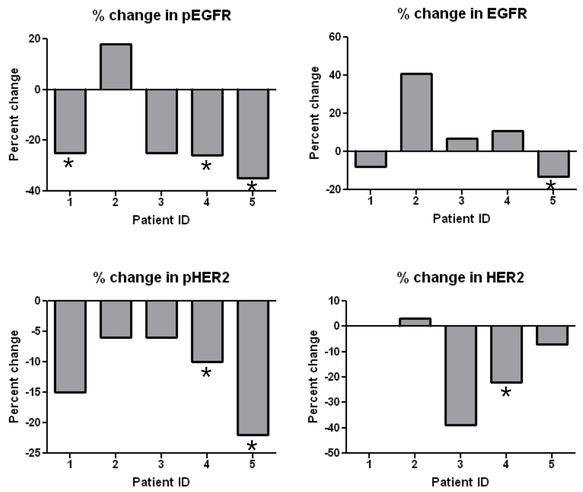

Figure 4. Significant reduction in EGFR (3 out of 5 patients) and HER2 (2 out of 5 patients) phosphorylation was observed in CTCs isolated from cancer patient blood treated *ex vivo* with CUDC-101. Percent change was calculated relative to DMSO treated control. *, p<0.05.

Significant increase in acetylated histone H3 protein levels in CTCs from cancer patient blood treated *ex vivo* with CUDC-101

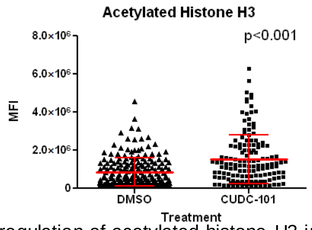

Figure 5. Up-regulation of acetylated histone H3 in CTCs. Each dot represents one CTC

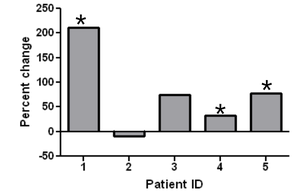

Figure 6. Significant increase in acetylated histone H3 was observed in the *ex vivo*-treated CTCs of patients 1, 4 and 5.

Conclusions

•CUDC-101 at 10 µM was effective in reducing EGFR and HER2 phosphorylation and increasing the accumulation of acetylated histone H3 in SCC-9 and SCC-15 head and neck cancer cell lines.

•CTCs isolated from cancer patient blood treated *ex vivo* with CUDC-101 (10 µM) showed reduced phosphorylated EGFR and phosphorylated HER2 and increased acetylated histone H3.

•Overall, CUDC-101 was shown to successfully inhibit EGFR, HER2 and HDAC pathways in CTCs isolated from *ex vivo* treated cancer patient blood.

Acknowledgement

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