1425 THE NOVEL SMALL MOLECULE MULTITARGET INHIBITOR LCI133 SENSITIZES ENDOMETRIAL CARCINOMA TO TRAIL-MEDIATED APOPTOSIS

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Background Endometrial carcinoma (EC) is the most common gynecological malignancy in the developed world.[1] While early-stage EC is easily treated, late-stage and recurrent disease have poor prognoses.[2] Targeted and immunotherapy options are limited and combination surgery, chemo-, and radiotherapy remains standard of care. Thus, an urgent unmet medical need exists to develop novel therapies that may potentiate or synergize with immunotherapy. Several groups previously showed that EC tumors and cell lines are resistant to TNF-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis.[3–6] We recently described the direct in vitro antitumor activity of novel multitarget small molecule chemotype LCI133, a nanomolar potent cyclin-dependent kinase (CDK) 4/6/9 inhibitor, in EC.[7 8] Here, we report that nanomolar concentrations of LCI133 overcome TRAIL resistance through CDK9 inhibition and dose-dependently sensitize EC cell lines to TRAIL-mediated apoptosis.

Methods To assess the effect of LCI133 on EC TRAIL sensitivity, we used pharmacologic and genetic drug screening methods to evaluate potency across multiple EC and stromal cell lines. IC_{50} was determined using CellTiter Glo Assay, apoptosis was assessed by flow cytometric detection of Annexin V/7-AAD, and gene and protein expression was characterized at the indicated timepoints by qRT-PCR and immunoblot.

Results EC cell lines displayed minimal sensitivity to TRAIL alone but LCI133 (nM) pre-treatment dose-dependently sensitized EC to TRAIL-mediated apoptosis. LCI133 ($\geq$0.250μM)-TRAIL combination induced apoptosis in nearly all cells at 24h as detected by Annexin V-7-AAD staining (figure 1A). Combination LCI133+TRAIL induced apoptosis as early as 4h and sustained for 24h. This phenotype was associated with significant increases in cleaved PARP and truncated-BID (tBID) as well as potent suppression of anti-apoptotic MCL-1. Furthermore, treatment with LCI133 (0.5μM) for 24h significantly suppressed *cFLIPS/L* transcription (P≤0.0001). Inhibition of caspase 3 or 8 potently attenuated combination LCI133-TRAIL-mediated apoptosis while caspase 9 inhibition had partial effect. Importantly, surface immunostaining revealed constitutive expression of TRAIL receptor 2/DR5 and minimal expression of TRAIL receptor 1/DR4 or decoy receptors DcR1/2. Short-term LCI133 treatment did not alter surface expression of DR4/5 (figure 1D). Further, LCI133 displayed a lower toxicity profile against non-transformed cells compared to single agent CDK9 inhibitor control.

Conclusions Together, these data cast a new light on the clinical failure of TRAIL therapy and provide evidence for its utility in cancer.[9] Additionally, it underscores the need to fully evaluate targeted therapy mechanism of action and explore the ability of epigenetic regulator and kinase inhibitors to mitigate immune escape and potentiate therapeutic response.

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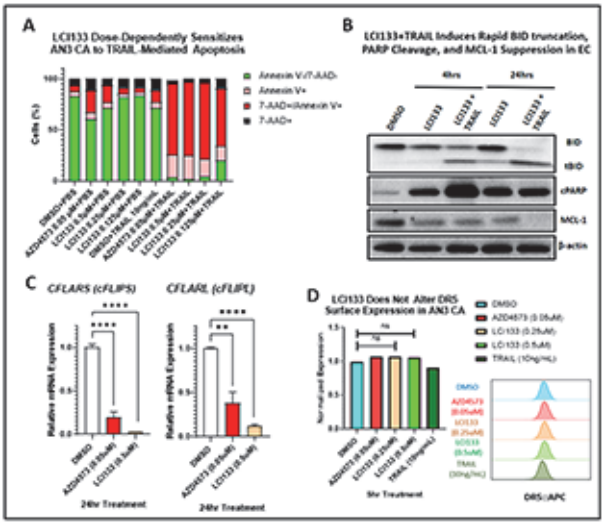
Ethics Approval All biohazard and hazardous agent work was approved by Atrium Health Institutional Biosafety Committee (IBC) and murine studies approved by Atrium Health Wake Forest Baptist under protocols A22–206, A23–015, and A23–032.



Abstract 1425 Figure 1 The novel small molecule multitarget inhibitor LCI133 sensitizes endometrial carcinoma to TRAIL-mediated apoptosis. (A) Apoptotic effect following 1h drug pretreatment and 24h TRAIL exposure as assessed by flow cytometry Annexin V/7-AAD staining. (B) Western blot analysis of apoptoss pathway protein expresson at the indicated timepoints following LCI133 (0 5µM) alone or TRAIL (10ng/mL) combination. (C) CFLAR Isoform expression following 24hr drug treatment as assessed by qRT PCR. (D) DR5 surface expression detected by flow cytometric staining of DR5::ÄPC (Biolegend, clone DJR-4 (7–8)) following 5h drug exposure

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