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Presentation Abstract

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Presentation Title:	Cyclin E amplification predicts sensitivity of primary Uterine Serous Carcinoma (USC) cell lines to the cdk2 inhibitor CYC065
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Abstract Body:	We evaluated the in vitro effectiveness of the cdk2 inhibitor CYC065 on multiple primary chemotherapy-resistant USC cell lines with or without CCNE1 amplification. CCNE1-amplified primary cell lines were significantly more sensitive than wild type USC cell lines to CYC065 in vitro (i.e., IC50: mean±STDV= 61.75±13.22 nM and 103.16± 21.9 nM for CCNE1-amplified USC-ARK-2 and USC-ARK-7 cell lines, respectively and 539.2±182.1 nM for the wild type USC-ARK-4 cell line, p=0.0048). Consistently, low concentrations of CYC065 (i.e., 100 - 300 nM) caused a dose dependent arrest in the G1 phase of the cell cycle specifically in CCNE1-amplified primary USC cell lines. Importantly, CCNE1 knockdown in the USC-ARK-2 cell line resulted in a 9.29-fold increase in the CYC065 IC50 when compared to the MOCK-transfected USC-ARK-2 cell line (p=0.021). Finally, when primary CCNE1-amplified USC cell lines also harboring ERBB2 amplification (50% of CCNE1-amplified USC cell lines) were incubated in vitro with the combination of CYC065 and Herceptin (a monoclonal antibody targeting the product of the ERBB2 gene, HER2/neu), an increased inhibitory effect was reported in the combination treatment when compared to Herceptin or CYC065 used as single agent (i.e., % viable cells: mean±STDV=71.4±0.85, 65.4±14.2, 42.2±2.1 for the Herceptin, the CYC065 and the combination treatment on USC-ARK-2, respectively; p=0.014). Together these findings identify CYC065 as a promising drug to be considered alone or in combination in the treatment of patients harboring CCNE1-amplified USC.

Cyclin E amplification predicts sensitivity of primary Uterine Serous Carcinoma (USC) cell lines to the cdk2 inhibitor CYC065

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INTRODUCTION

Uterine serous carcinoma (USC) is an aggressive variant of endometrial cancer. Although it accounts for less than 10% of all endometrial tumors, it causes up to 39% of all endometrial cancer-related deaths. USC, referred to as a Type II endometrial cancer, is characterized by a high-grade, complex histology and carries a poor prognosis as it is often spread beyond the uterine corpus at the time of diagnosis[1]. Thus, there is a dire need for a better understanding of its molecular response to potential therapies in order to develop novel, target-specific and more effective therapeutic strategies against this rare subset of endometrial cancer.

We have recently evaluated the genetic landscape of a large number of USC and we found that alterations in the cell cycle pathway occurred in up to 88% of these tumors. In particular the amplification of the gene encoding for Cyclin E1 (CCNE1), an activator of Cyclin-dependent kinase 2 (Cdk2), was reported in 48% of the tumor samples.

Here, we have evaluated the effectiveness of the Cyclin-dependent kinase 2 (Cdk2) and 9 (Cdk9) inhibitor CYC065 (Cyclacel Ltd, Dundee, UK. Saladino et al. AACR2015 Abs#1650) in vitro and in vivo on multiple CCNE1-amplified primary chemotherapy-resistant USC cell lines and USC-derived xenograft mouse models.

RESULTS and CONCLUSION

CCNE1-amplified primary USC cell lines were significantly more sensitive than wild type to CYC065 *in vitro* (i.e., IC50: mean±STDV= 124.12±57.8 and 415±117.5 nM for CCNE1-amplified and wild type cell lines, respectively, *p=0.0003*). Consistently, low concentrations of CYC065 (i.e., 100 nM) caused an arrest in the G1 phase of the cell cycle specifically in CCNE1-amplified primary USC cell lines. Importantly, CCNE1 knockdown resulted in a 9.29 fold increase in the IC50 (*p=0.021*). In vivo data showed that the treatment of CCNE1-amplified USC-derived xenografts with CYC065 significantly reduced tumor growth compared to vehicle treated mice (*p=0.012* starting at day 9 of the treatment). Importantly, when CCNE1-amplified primary USC cell lines also carrying hyperactivation of the PI3CA/AKT/mTOR-signaling pathway (50% of CCNE1-amplified USC cell lines) were incubated *in vitro* with the combination of CYC065 and Herceptin (a monoclonal antibody targeting the product of the c-erBB2 gene, HER2/neu) or CYC065 and the PIK3CA inhibitor Taselisib, a synergistic effect was reported in the combination treatments when compared to each of the single drugs (CI=ED50/ED75/ED90<1). Consistently, when the same cell lines were used to establish xenografts in mice, combination treatments were significantly more effective in reducing tumor growth compared to the single agents (*p<0.05*).

Together these findings identify CYC065 as a promising drug to be considered alone or in combination in the treatment of patients harboring CCNE1-amplified USC.

CCNE1 amplification and mRNA expression in USC

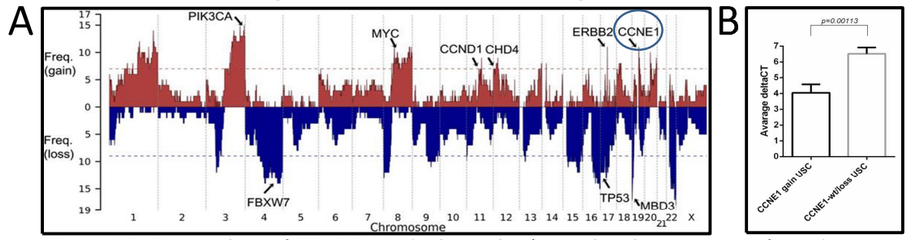

Figure 1.A: CNV analysis of 25 USC matched samples (normal and tumor tissue from the same patient) showing genetic somatic amplifications and deletions. CCNE1 amplification was found in 48% of the samples. B: RT-PCR data on the 25 USC samples analyzed for CNV. CCNE1-amplified USC express significantly higher levels of CCNE1 mRNA compared to wt tumors (*p=0.00113*).

CCNE1 expression in selected Primary USC cell lines

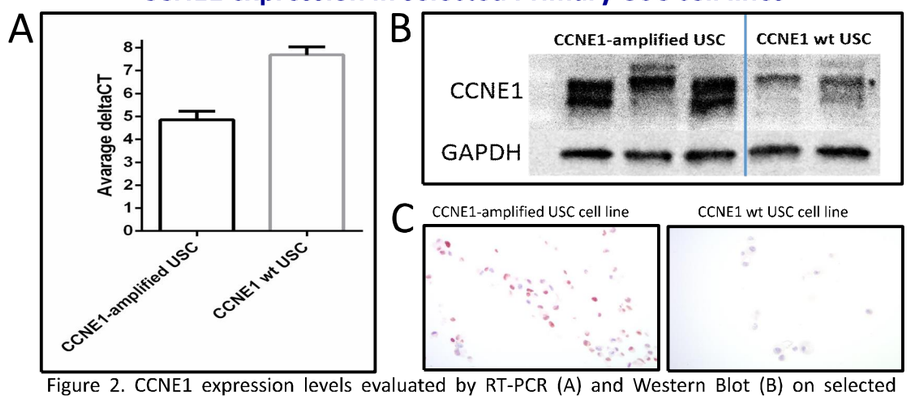

Figure 2. CCNE1 expression levels evaluated by RT-PCR (A) and Western Blot (B) on selected primary USC cell lines. CCNE1-amplified USC express significantly higher levels of CCNE1 mRNA and protein than wt USC cell lines (*p<0.05*). C: Representative CCNE1 immunostaining images of a CCNE1-amplified and a wt USC cell line.

In vitro efficacy of CYC065 on primary USC cell lines

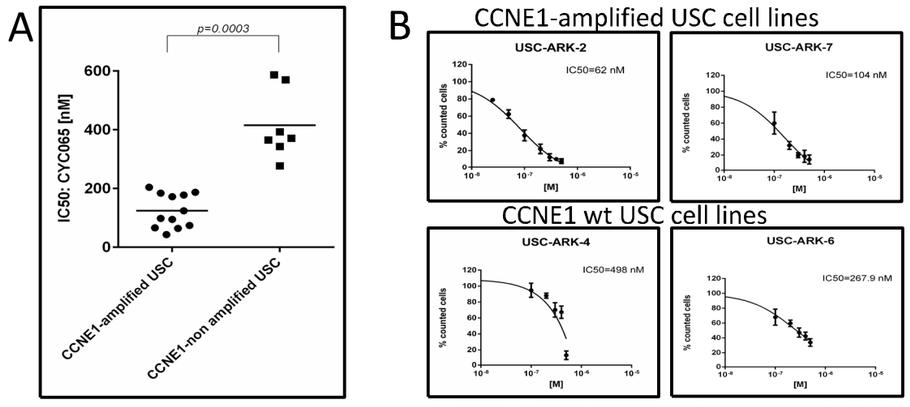

Figure 3. A: in vitro sensitivity of selected primary USC cell lines to the Cdk2 inhibitor CYC065. CCNE1-amplified USC cell lines are significantly more sensitive than wt USC cell lines to CYC065 (IC50: mean±STDV= 124.12±57.8 and 415±117.5 nM for CCNE1-amplified and wt cell lines, respectively, p=0.0003). B: Representative dose-response curves of CCNE1-amplified (upper panel) and wt (lower panel) USC cell lines treated with scalar doses (ranging from 100 to 600nM) of CYC065 for 72 hours.

CYC065 specificity and *in vivo* activity as single agent

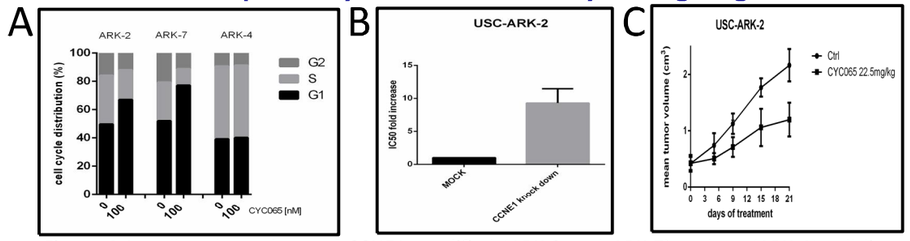

Figure 4.A: cell cycle analyses of CCNE1-amplified (ARK-2 and ARK-7) and wt (ARK-4) cell lines treated with 100nM of CYC065 for 48 hours. CYC065 caused an arrest in the G1 phase of the cell cycle specifically in CCNE1-amplified USC. B: Knockdown of CCNE1 resulted in a 9.29 fold increase in the IC50 (*p=0.021*). C: The daily (7d/week)*3 treatment of CCNE1-amplified USC-derived xenografts with 22.5mg/kg of CYC065 significantly reduced tumor growth compared to vehicle treated mice (*p=0.012* starting at day 9 of the treatment).

CYC065 in combination with Herceptin on CCNE1/c-erbB2 amplified USC

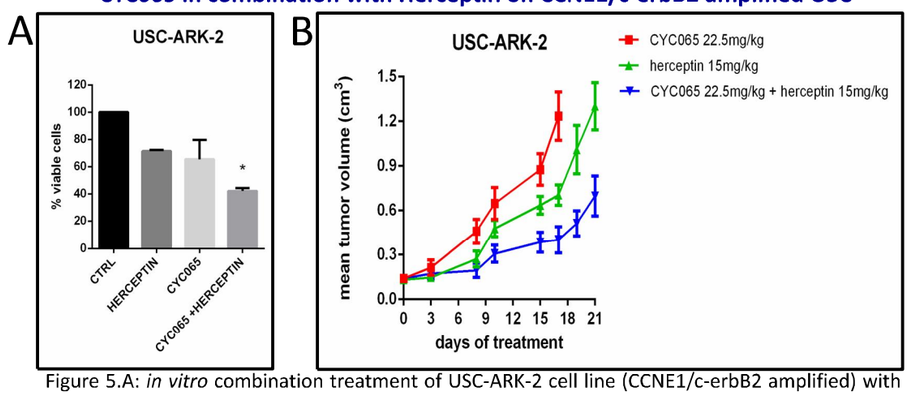

Figure 5.A: *in vitro* combination treatment of USC-ARK-2 cell line (CCNE1/c-erbB2 amplified) with Herceptin and CYC065. Combination of drugs has a synergistic effect on cell growth inhibition (CI calculated using CompuSyn software=ED50/ED75/ED90<1). B: in vivo treatment of USC-ARK-2-derived xenografts with Herceptin (1d/week) *3 and CYC065 (7d/week)*3 significantly reduced tumor growth compared to each of the single drugs (Herceptin versus combo *p=0.0212* at day 15; CYC065 versus combo *p=0.016* at day 8).

CYC065 in combination with Taselisib on CCNE1-amplified PIK3CA-mutated USC

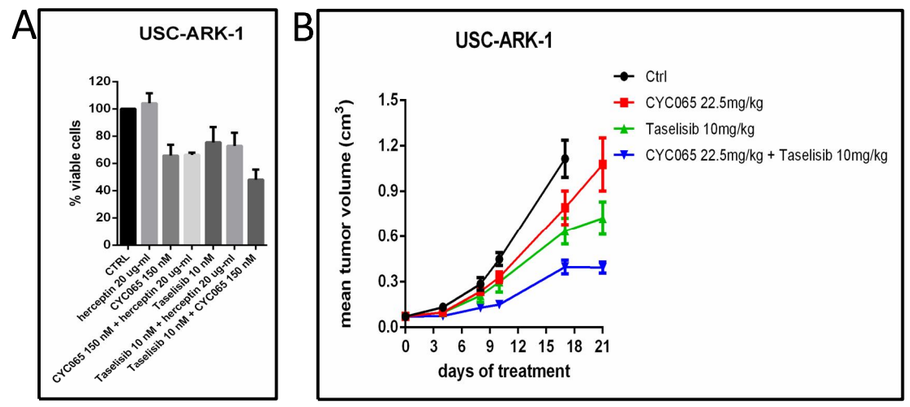

Figure 6.A: *in vitro* combination treatment of USC-ARK-1 cell line (CCNE1-amplified PI3KCA mutated) with Taselisib and CYC065. Combination of drugs has a synergistic effect on cell growth inhibition (CI calculated using CompuSyn software=ED50/ED75/ED90<1). B: in vivo treatment of USC-ARK-1-derived xenografts with Taselisib (5d/week)*3 and CYC065 (7d/week)*3 significantly reduced tumor growth compared to each of the single drugs (Taselisib versus combo *p=0.043* at day 10: CYC065 versus combo *p=0.037* at day 4).