526P: Pharmacodynamic evidence for WEE1 target engagement in surrogate and tumor tissues from a Phase 1 study of the WEE1 inhibitor ZN-c3

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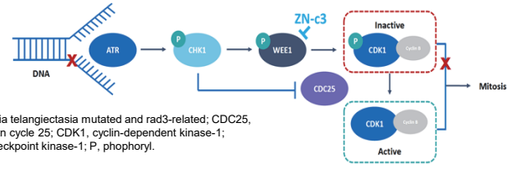
Introduction

- WEE1 tyrosine kinase is a critical component of the G2/M cell cycle checkpoint that phosphorylates CDK1 (p-CDK1), inducing cell cycle arrest in the presence of DNA damage to allow for DNA repair.
- Inhibition of WEE1 can induce mitotic catastrophe and apoptosis.[1]
- ZN-c3 is a novel selective inhibitor of WEE1 (**Figure 1**).[2]

Figure 1. Mechanism of Action for ZN-c3



ATR, ataxia telangiectasia mutated and rad3-related; CDC25, cell division cycle 25; CDK1, cyclin-dependent kinase-1; CHK1, checkpoint kinase-1; P, phophoryl.

Methods

- ZN-c3 was tested in the A-427 xenograft model of non-small cell lung cancer (NSCLC).
- Assays using immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) tissues were developed for p-CDK1, γH2AX, and Ki-67.
- In a Phase 1 clinical trial, ZN-c3 is being evaluated in subjects with advanced or metastatic solid tumors (**Figure 2**).[3]
- A maximum tolerated dose of 350 mg once daily, and a recommended phase 2 dose (RP2D) of 300 mg once daily, have been identified.[3]
- Skin biopsy specimens were obtained at baseline and after 15 days of treatment in 23 patients treated at RP2D or higher; biopsy specimens of tumor tissue were obtained at baseline and after 21 days of treatment in 3 patients treated at RP2D or higher.

Figure 2. Study Design

- ≥18 years old
- Solid tumor with advanced or metastatic disease, refractory to standard therapy or for whom no standard therapy is available, or the subject is ineligible for standard therapy(ies)
- Adequate hematologic and organ function
- ECOG Performance Status ≤2
- Measurable or evaluable disease per RECIST v1.1

Dose Expansion

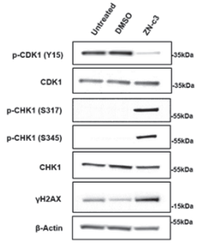
Dose Expansion with subjects with uterine serous carcinoma (USC) and Mutations of Interest (N = ~40)

ECOG, Eastern Cooperative Oncology Group; MTD, maximum tolerated dose; RECIST, Response Evaluation Criteria in Solid Tumors.

Results

Figure 3: WEE1 pathway modulation *in vitro*

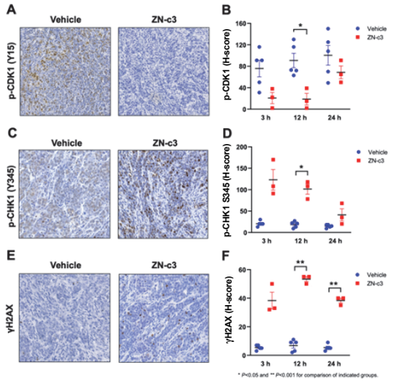
- A-427 NSCLC cells were treated with 150 nM ZN-c3 for 4 hours and analyzed by Western blotting (**Figure 3**).
- Inhibition of downstream phosphorylation target CDK1 (Y15) and enhanced phosphorylation of upstream CHK1 (S317 and S345, respectively) were observed.
- ZN-c3 treatment also resulted in enhanced levels of DNA damage marker γH2AX.



Study sponsored by K-Group Alpha, Inc, a subsidiary of Zentalis Pharmaceuticals, Inc.; NCT04500587.

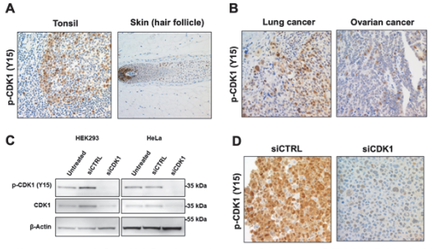
Results (continued)

Figure 4. Effects of ZN-c3 in mouse tumor tissue



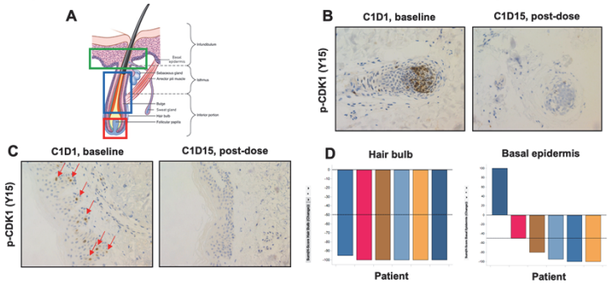
- Nonobese diabetic/severe combined immunodeficiency mice were inoculated with 1 × 10^7 A-427 cells in the flank.
- After tumors reached ~350 mm^3 in size, mice received vehicle or ZN-c3 (80 mg/kg QD) orally for 3 days and then were euthanized.
- **Figure 4** shows staining in tumor tissue, and averaged H-scores of 3 fields of interest determined by a board-certified pathologist for p-CDK1 (A and B), p-CHK1 (C and D), and γH2AX (E and F) The results show inhibition of p-CDK1 and enhanced levels of p-CHK1 and γH2AX, in agreement with **Figure 3**.

Figure 5. Validation of p-CDK1 by IHC in human tissue



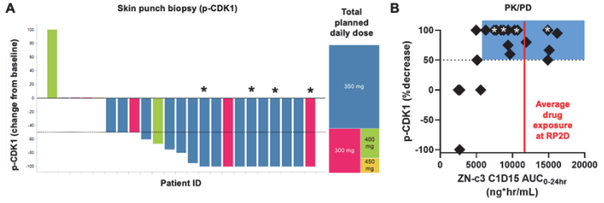
- Of 3 antibodies tested for IHC, the clone of anti-p-CDK1 (Y15) (CS4539S) antibody was selected based on the expected nuclear staining pattern in proliferating cells, with higher selectivity and specificity in a wide variety of tissues.
- Specific p-CDK1 staining patterns are shown for normal human tonsil and skin (**Figure 5A**), and for lung and ovarian cancer (**Figure 5B**).
- In addition, siRNA-mediated knockdown of CDK1 in HEK293 and HeLa cells was performed (**Figure 5C**) and the FFPE cell pellets showed significantly reduced immunoreactivity for p-CDK1 (**Figure 5D**).

Figure 6. p-CDK1 expression in skin samples and scoring



- In human skin samples, hair follicle (hair bulb = red, isthmus = blue) and basal epidermis (= green) sections were scored semiquantitatively for p-CDK1 (**Figure 6A**).
- Hair bulbs showed the largest dynamic range in p-CDK1 reactivity (**Figure 6B**).
- However, intact hair bulbs in paired (baseline vs on-treatment) samples were identified in only 9 of 23 patients, whereas p-CDK1 scoring in the basal epidermis (example in **Figure 6C**) could be performed for all 23 patients.
- Changes in p-CDK1 in hair bulbs correlated with changes in basal epidermis: 6 of 9 patients showed ≥50% decreases in p-CDK1 reactivity (H-score) from baseline, and 5 of these 6 also showed p-CDK1 decreases of ≥50% in the basal epidermis (**Figure 6D**).

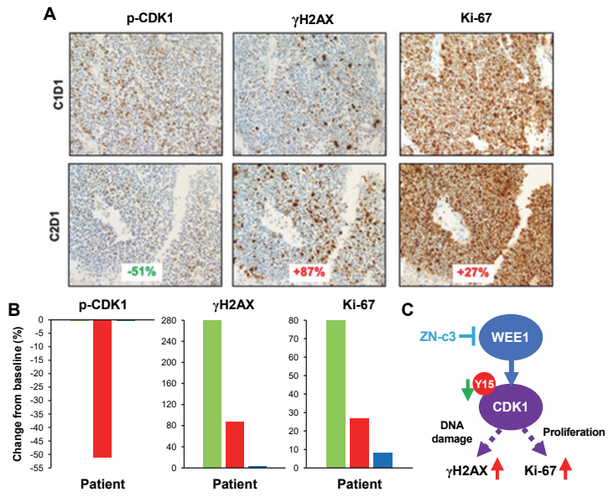
Figure 7. Pharmacodynamic (PD) and Pharmacokinetic (PK)/PD in skin punch biopsy specimens



A. Semiquantitative scoring of p-CDK1 represented as change in H-score at Cyle 1, Day 15 (C1D15) 3-4 hours post-dose, relative to baseline (Cycle 1, Day 1 [C1D1], pre-dose). The total planned daily doses are color coded; patients with confirmed partial response (cPR) are indicated with an asterisk (*). **B.** PK/PD relationship showing plasma levels of ZN-c3 (AUC_{0-24h}) versus p-CDK1 decrease (%) on C1D15. Patients with cPR are indicated with an asterisk. Average drug exposure at RP2D is indicated by the red line.

- 18 of 23 patients (78%) showed ≥50% inhibition of p-CDK1 in skin biopsies after ZN-c3 treatment. Furthermore, all patients with cPR (n = 4) showed evidence of target engagement (**Figure 7A**).
- For all patients above the AUC_{0-24h} threshold (~6200 ng*hr/mL), WEE1 pathway modulation was detected in skin biopsy specimens (**Figure 7B**).
- These data confirm on-target effects of ZN-c3 and support the RP2D.
- Of note, proliferation marker Ki-67 and DNA damage marker γH2AX did not demonstrate consistent upregulation or downregulation in the skin.

Figure 8. Changes in PD markers in biopsy specimens of tumor tissue



A. Examples of p-CDK1, γH2AX, and Ki-67 staining in tumor tissue from a patient at baseline (C1D1) and on treatment (Cycle 2, Day 1 [C2D1], 2 hours post-dose). **B.** Changes in PD markers in tumor tissue from 3 patients (color coded). **C.** Schematic of predicted changes of biomarkers in tumor tissue.

- Tumor tissue was collected at baseline and during treatment from 3 patients treated at RP2D or higher. One of these patients showed significantly decreased p-CDK1 levels on treatment (shown in red in **Figure 8B**), whereas the other 2 showed evidence of downstream pathway modulation (increased γH2AX and Ki-67).
- These data confirm on-target effects of ZN-c3 in the target tissue (tumor), including increased biomarkers of proliferation and DNA damage consistent with the mechanism of WEE1 inhibition.

Conclusions

- Target engagement by WEE1 inhibitor ZN-c3 was demonstrated in skin and tumor tissue from patients treated at the RP2D or higher in a Phase 1 trial.
- PK/PD analysis confirmed a correlation between ZN-c3 plasma levels and WEE1 pathway modulation.

References

1. Matheson CJ, et al. *Trends Pharmacol Sci.* 2016;37(10):872-881. **2.** Li J. (April 2021). Poster presentation, abstract 1965. AACR, virtual. **3.** Tolcher A. (April 2021). Oral presentation, abstract CT016. AACR virtual.

Disclosure

Dr Chalasani: Advisory boards for Zentalis, Eli Lilly, OncoSec, and AstraZeneca; research funding from Pfizer.