Poster #4373

# Discovery of ZN-c5 a Novel Potent and Oral Selective Estrogen Receptor Degrader

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## Background

The majority of breast cancers express estrogen receptor alpha (ERα), and the two major strategies for therapeutic targeting of ERα-positive breast cancer are aromatase inhibition and inhibition of ER signaling. Although these strategies can be effective, many patients develop resistance, which ultimately leads to disease progression that continues to rely on estrogen receptor signaling.

Fulvestrant, a small molecule ER antagonist, is the only selective estrogen receptor degrader (SERD) that is approved for the treatment of ER+/HER2– metastatic breast cancer. Although it is effective, it is limited by its poor bioavailability and administration by intramuscular injection, resulting in sub-optimal drug exposure.

Here we describe ZN-c5, a novel small molecule with potent antagonism and degradative properties against the estrogen receptor, that demonstrates high oral bioavailability.

## Results

Table 1 presents ERα binding affinity and proliferation inhibition values for ZN-c5 and comparator ER antagonists.

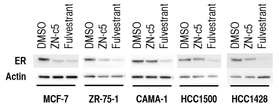
**Table 1. ERα Binding Affinity and Proliferation Inhibition Values for ZN-c5 and Competitors**

| Compound | ERα Binding $IC_{50}$ (nM) | MCF-7 $IC_{50}$ (nM) |
|---|---|---|
| Fulvestrant | 2.1 | 0.7 |
| RAD1901 | 0.59 | 0.3 |
| AZD9496 | 0.22 | 0.1 |
| LSZ-102 | 0.15 | 0.5 |
| SAR439859 | 1.0 | 0.9 |
| **ZN-c5** | **0.40** | **0.3** |

Proliferation Assay. MCF-7 cells were grown in phenol red free DMEM/F12 media containing 8% charcoal-stripped FBS then treated with 1nM beta-estradiol and indicated ER antagonist for 6 days. Cell viability measured using Cell Titer-Glo assay.

Figure 1 presents the results of the ERα Western Blot Analysis, comparing the ER degradation induced in breast cancer cell lines by ZN-c5 with that of DMSO and fulvestrant. Immunoblotting was performed to determine ERα levels using whole cell lysates.

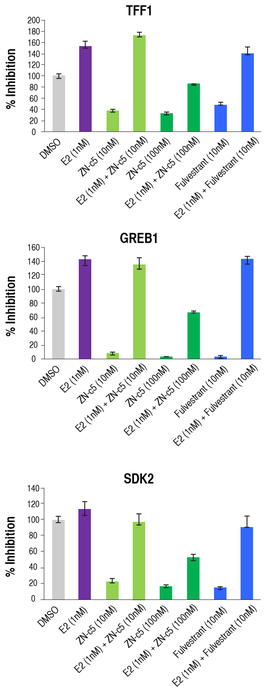
### Figure 1. ERα Degradation by ZN-c5 in Breast Cancer Cell Lines




ERα Western Blot Analysis. Cells were grown in phenol red free DMEM/F12 media supplemented with 8% charcoal-stripped FBS, non-essential amino acids and sodium pyruvate for 48 hours before treatment with 100nM of indicated ligands for 24 hours. Immunoblotting was performed to determine ERα level using whole cell lysates.

Figure 2 shows the decrease in transcription of selected ERα target genes, TFF1, GREB1, and SDK2, with ZN-c5 and comparators.

### Figure 2. Decreased Transcription of Selected ERα Target Genes by ZN-c5




MCF-7 cells were grown in DMEM/F12 media containing 8% charcoal-stripped FBS and then treated with indicated ligands (indicated concentrations) for 24 hours prior to RNA isolation and gene expression analysis by qPCR.

Table 2 displays the pharmacokinetic properties of ZN-c5 when dosed 10 mg/kg, once daily, in mice, rats, monkeys and dogs.

These nonclinical in vivo data indicate that ZN-c5 is rapidly absorbed, with oral bioavailability typically exceeding 74% in all species.

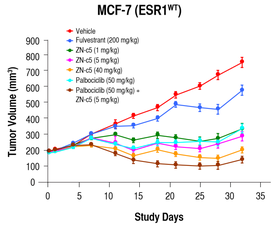
**Table 2. Pharmacokinetic Properties of ZN-c5 in Multiple Species**

| | Mouse | Rat | Monkey | Dog |
|---|---|---|---|---|
| Oral Administration | PO | | | |
| Dose (mg/kg) | 10 | | | |
| $AUC_{0\text{-}inf}$ (μg*hr/mL) | 28.4 | 123 | 26.3 | 477 |
| $C_{max}$ (μg/mL) | 5.02 | 14.5 | 6.93 | 29.8 |
| F(%) Bioavailability | 74 | 88 | 133 | 88 |
| $T_{max}$ | 0.5 | 1 | 1 | 2 |
| IV Administration | IV | | | |
| Half-Life $t_{1/2}$ (hrs) | 2.9 | 4.2 | 5.6 | 21.2 |

Mice, rats, monkeys and dogs were dosed once orally.
Plasma samples were collected at 0.5, 1, 2, 4, 8, 12 and 24 hours post dose.

To test whether its high oral bioavailability can be translated to potent efficacy in vivo, we evaluated the antitumor activity of ZN-c5 in the MCF-7 orthotopic tumor xenograft model. As shown in Figures 3 and 4, ZN-c5 treatment results in robust antitumor activities in MCF-7 tumor xenografts as a single agent and in combination with palbociclib.

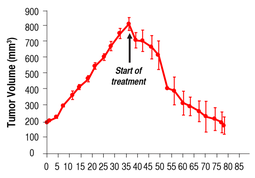
### Figure 3. Robust Antitumor Activities of ZN-c5 in MCF-7 Tumor Xenografts as a Single Agent and in Combination with Palbociclib




BALB/c nude mice bearing MCF-7 tumor cells were dosed orally once a day for 32 days. Fulvestrant was dosed subcutaneously once a week. All the animals in the study received an estradiol benzoate injection subcutaneously twice a week.

The combination of ZN-c5 (30 mg/kg) and palbociclib (50 mg/kg) induces regression, even in mice with large tumor burdens.

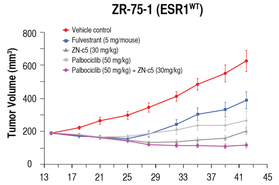
### Figure 4. Regression in Mice with Large Tumor Burden by ZN-c5 in Combination with Palbociclib




On day 36 mice with large tumor burden (vehicle group) were dosed with a combination of 50 mg/kg of palbociclib and 30 mg/kg of ZN-c5.

As presented in Figure 5, ZN-c5 demonstrates improved antitumor efficacy in the ZR-75-1 xenograft model, compared with fulvestrant or palbociclib.

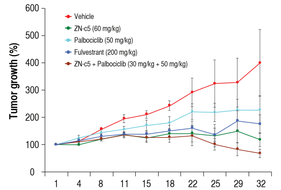
### Figure 5. Significant Antitumor Efficacy of ZN-c5 in ZR-75-1 Xenograft Model




BALB/c nude mice bearing ZR-75-1 tumors were orally dosed with ZN-c5, palbociclib or with a combination of palbociclib and ZN-c5 once a day for 28 days. Fulvestrant was dosed subcutaneously once a week. All the animals in the study received estradiol benzoate injection subcutaneously twice a week. All doses were tolerated.

In the HCI-005 ESR1 mutant PDX model, ZN-c5 demonstrated significant efficacy as a single agent, and increased antitumor activity in combination with palbociclib (Figure 6).

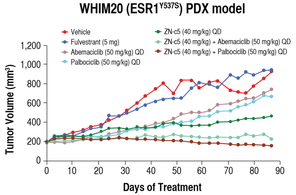
### Figure 6. Significant Efficacy of ZN-c5 as a Single Agent and Increased Antitumor Activity in Combination with Palbociclib in HCI-005 ESR1 Mutant PDX Model




NSG mice bearing HCI-005 PDX tumors were orally dosed ZN-c5, palbociclib or with a combination of palbociclib and ZN-c5 once a day for 31 days. Fulvestrant was dosed subcutaneously once a week. All the animals in the study were supplemented with estradiol benzoate. All doses were tolerated.

We also evaluated the activity of ZN-c5 in the ER mutant model, WHIM20, a Y537S ESR1 patient-derived xenograft model. As shown in Figure 7, ZN-c5, 40 mg/kg once daily, demonstrated improved inhibition of tumor growth as a single agent, compared with fulvestrant, and resulted in enhanced antitumor activity in combination with CDK4/6 inhibitors in this human xenograft model. All doses were tolerated.

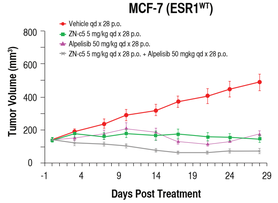
### Figure 7. Significant Efficacy of ZN-c5 as a Single Agent and Increased Antitumor Activity in Combination with Palbociclib or Abemaciclib in WHIM20 PDX Model




Nude mice bearing WHIM20 PDX tumors were orally dosed once a day for 90 days. Fulvestrant was dosed subcutaneously once a week. All doses were tolerated.

The combination of ZN-c5 with alpelisib also enhanced tumor growth inhibition in the MCF-7 xenograft model (Figure 8).

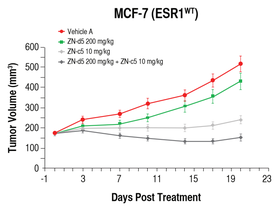
### Figure 8. Enhanced Tumor Growth Inhibition by the Combination of ZN-c5 with Alpelisib in MCF-7 Xenograft Model




BALB/c nude mice bearing MCF-7 tumor cells were dosed orally once a day for 28 days. All the animals in the study received estradiol benzoate injection subcutaneously twice a week. All doses were tolerated.

Using the same model, the combination of ZN-c5 with Zentalis's BCL-2 inhibitor (ZN-d5) demonstrated increased tumor growth inhibition (Figure 9).

### Figure 9. Increased Tumor Growth Inhibition by the Combination of ZN-c5 with Zentalis's BCL-2 Inhibitor (ZN-d5) in MCF-7 Xenograft Model




BALB/c nude mice bearing MCF-7 tumor cells were dosed orally once a day with vehicle, ZN-c5, ZN-d5 or combination of ZN-c5 and ZN-d5 for 21 days. All the animals in the study received an estradiol benzoate injection subcutaneously twice a week. All doses were tolerated. ZN-d5 is a novel oral selective inhibitor of BCL-2.

Figure 10 and Table 3 present the human mean plasma PK parameters at the 50 mg dose of ZN-c5.

### Figure 10. Human Mean Plasma PK Parameters of ZN-c5 at 50 mg Dose

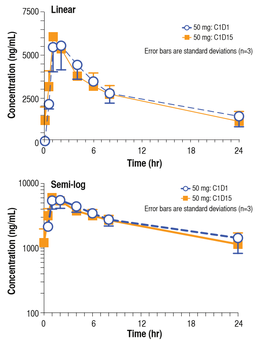



**Table 3. Human Mean Plasma PK Parameters of ZN-c5 at 50 mg Dose**

| | Day 1 | | | Day 15 | | | D15/D1 AUC Ratio |
|---|---|---|---|---|---|---|---|
| | $C_{max}$ (μg/mL) | $T_{max}$ (hr) | $AUC_{0\text{-}24hr}$ (μg*h/mL) | $C_{max}$ (μg/mL) | $T_{max}$ (hr) | $AUC_{0\text{-}24hr}$ (μg*h/mL) | |
| **Mean** | **5.73** | **2** | **65.7** | **5.81** | **1** | **61.3** | **0.94** |
| **SD** | *1.33* | *1-2* | *7.35* | *0.405* | *1-2* | *10.4* | *0.20* |
| **CV (%)** | 23.3 | | 11.2 | 6.97 | | 17.6 | 21.4 |

## Conclusions

- ZN-c5 is a novel potent antagonist of ERα, that inhibits the proliferation of ER+ breast cancer cells and induces ERα degradation.
- In ESR1 wild-type and ESR1 mutant breast cancer xenograft models, ZN-c5 caused tumor growth inhibition as a single agent. In combination treatments, ZN-c5 enhanced tumor growth inhibition when combined with CDK4/6 inhibitors, PI3K inhibitor or BCL-2 inhibitor.
- ZN-c5 showed improved pharmaceutical properties, including high oral bioavailability across several preclinical species.
- The pharmacokinetic profile of ZN-c5 in breast cancer patients showed that ZN-c5 has greater exposure than other SERDs that are currently in clinical development.
- The high exposure of ZN-c5, coupled with its potency and degradative properties, could therapeutically benefit estrogen receptor-positive breast cancer patients.
- Phase 1/2 clinical trials of ZN-c5 are ongoing.

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