Correlation of Cyclin E1 Expression and Clinical Outcomes in a Phase 1b Dose-Escalation Study of Azenosertib (ZN-c3), a WEE1 inhibitor, in Combination with Chemotherapy (CT) in Patients (pts) with Platinum-Resistant or Refractory (R/R) Epithelial Ovarian, Peritoneal, or Fallopian Tube Cancer (EOC)

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INTRODUCTION

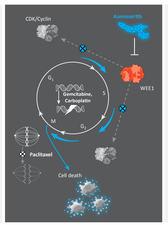
- Azenosertib is an oral, highly specific, potent inhibitor of WEE1 kinase. Preclinical and clinical data have shown it to be highly synergistic with multiple chemotherapies (**Figure 1**).
- Cyclin E1 positivity accelerates the G1/S transition, resulting in replication stress and rendering cells even more sensitive to WEE1 inhibition (**Figure 2**).
- Cyclin E1 positivity is also strongly correlated with platinum resistance and worse ovarian cancer outcomes (**Figure 3**).
- Zentalis 002 was a Phase 1b dose escalation study to define the recommended Phase 2 dose and early clinical activity of azenosertib in combination with chemotherapy.
- The purpose of this analysis was to describe the results of Zentalis 002 to date and determine if Cyclin E1 status was associated with benefit from azenosertib.

RESULTS

Figure 1: Multiple Chemotherapeutic Agents Induce DNA or Mitotic Machinery Damage, with Mechanistic Potential to Synergize with Azenosertib

Paclitaxel stops microtubules from disassembling in M phase causing mitotic catastrophe and cell death.

Azenosertib inhibits WEE1 and drives the cell cycle through G2/M thereby sensitizing cells to paclitaxel.



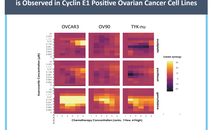
Carboplatin creates cross-linking DNA adducts, **Gemcitabine** causes DNA chain termination and fragmentation.

In response to chemotherapy-induced DNA damage, intact WEE1 arrests the cell cycle allowing for adequate DNA repair.

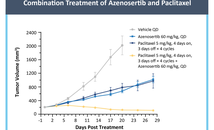
Azenosertib inhibits WEE1 and allows the cell cycle to proceed despite unrepaired chemotherapy-induced DNA damage, leading to cell death.

Figure 2: Synergy between Azenosertib and Chemotherapy in Non-Clinical *CCNE1* Amplified Ovarian Cancer Models

Increased Synergy between Azenosertib and Chemotherapy is Observed in Cyclin E1 Positive Ovarian Cancer Cell Lines



A Cyclin E1 Positive Tumor Model is Sensitive to Combination Treatment of Azenosertib and Paclitaxel



Ma J, et al. Cancer Res 2023;83(7_Supplement):2153.

Figure 3: Ovarian Cancer Patients with *CCNE1* Amplified and/or Cyclin E1 Positive Cancers have a Worse Outcome Following Platinum-Based Chemotherapy Treatment

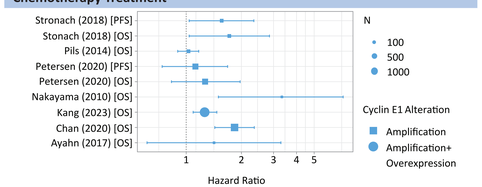


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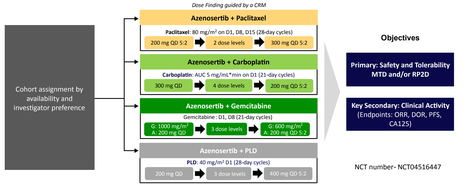
Figure 4: Zentalis 002 Trial Schema

Key Eligibility: High-Grade Serous Ovarian Cancer; ECOG performance status 0-2; Platinum-resistant/refractory; Up to 3 prior lines of chemotherapy; Measurable disease per RECIST v 1.1



Abbreviations: 5:2, 5-days of treatment followed by 2-days off treatment; A, azenosertib; AUC, area under the time-concentration curve; CRM, continuous reassessment model; DOR, duration of response; ECOG, Eastern Cooperative Oncology Group; G, gemcitabine; MTD, maximum tolerated dose; ORR, objective response rate; PFS, progression-free survival; PLD, pegylated liposomal doxorubicin; PROC, platinum-resistant ovarian cancer; RECIST, response evaluation criteria in solid tumors; RP2D, recommended Phase 2 dose.

Table 1: Baseline Characteristics

Characteristic		Azenosertib + Paclitaxel (N=26)	Azenosertib + Carboplatin (N=36)	Azenosertib + Gemcitabine (N=18)	Azenosertib + PLD (N=35)	Total (N=115)
Age	Median (Range)	61.5 (45-83)	61.0 (48-77)	62.5 (47-77)	56.0 (34-75)	61.0 (34-83)
Race and Ethnicity, n (%)	White	24 (92.3)	34 (94.4)	16 (88.9)	34 (97.1)	108 (93.9)
	Black or African-American	0	0	0	0	0
	Asian	1 (3.8)	1 (2.8)	1 (5.6)	1 (2.9)	4 (3.5)
	Other / NR	1 (3.8)	1 (2.8)	1 (5.6)	0	3 (2.6)
	Hispanic (Yes/No/NR)	1/25/0 (3.8/96.2/0)	0/34/2 (0/94.4/5.6)	1/17/0 (5.6/94.4/0)	1/33/1 (2.9/94.3/2.9)	3/109/3 (2.6/94.8/2.6)
ECOG Performance Status, n (%)	0	21 (80.8)	21 (58.3)	12 (66.7)	24 (68.6)	78 (67.8)
	1	5 (19.2)	15 (41.7)	6 (33.3)	11 (31.4)	37 (32.2)
Geographic Region, n (%)	US	6 (23.1)	10 (27.8)	10 (55.6)	5 (14.3)	31 (27.0)
	Europe	10 (38.5)	10 (27.8)	6 (33.3)	27 (77.1)	53 (46.1)
	Australia	9 (34.6)	15 (41.7)	1 (5.6)	3 (8.6)	28 (24.3)
	Korea	1 (3.8)	1 (2.8)	1 (5.6)	0	3 (2.6)
Platinum Status	Refractory, n (%)	5 (19.2)	9 (25.0)	3 (16.7)	7 (20.0)	24 (20.9)
Prior Lines of Therapy	1-2, n (%)	22 (84.6)	30 (83.3)	18 (100)	33 (94.3)	103 (89.6)
	3-4, n (%)	4 (15.4)	6 (16.7)	–	2 (5.7)	12 (10.4)
Prior PARP Inhibitor	n (%)	8 (30.8)	10 (27.8)	5 (27.8)	5 (14.3)	28 (24.3)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; NR, not reported; PLD, pegylated liposomal doxorubicin.

Table 2: Treatment-Related Adverse Events ≥20%

Treatment-Related Adverse Event, n (%)		Azenosertib + Paclitaxel (Continuous, N=7; Intermittent, N=19)		Azenosertib + Carboplatin (Continuous, N=22; Intermittent, N=14)		Azenosertib + Carboplatin (Continuous, N=14; Intermittent, N=8)		Azenosertib + Gemcitabine (Continuous N=8; Intermittent, N=10)		Azenosertib + PLD (Continuous N=27; Intermittent, N=8)		Total (Continuous, N=64; Intermittent, N=51)	
		All Doses*		All Doses		Doses ≤ MTD		All Doses**		All Doses*			
Grade		All Gr	Gr ≥3	All Gr	Gr ≥3	All Gr	Gr ≥3	All Gr	Gr ≥3	All Gr	Gr ≥3	All Gr	Gr ≥3
Hematologic	Neutropenia	C 5 (71.4)	5 (71.4)	9 (40.9)	7 (31.8)	4 (28.6)	3 (21.4)	7 (87.5)	6 (75.0)	19 (70.4)	17 (63.0)	40 (62.5)	35 (54.7)
		I 11 (57.9)	5 (26.3)	7 (50.0)	1 (7.1)	4 (50.0)	-	7 (70.0)	4 (40.0)	3 (37.5)	3 (37.5)	28 (54.9)	13 (25.5)
	Thrombo-cytopenia	C 4 (57.1)	2 (28.6)	16 (72.7)	11 (50.0)	11 (78.6)	6 (42.9)	8 (100.0)	5 (62.5)	9 (33.3)	2 (7.4)	37 (57.8)	20 (31.3)
		I 4 (21.1)	-	9 (64.3)	5 (35.7)	4 (50.0)	2 (25.0)	8 (80.0)	6 (60.0)*	3 (37.5)	3 (37.5)	24 (47.1)	14 (27.5)
	Anemia	C 5 (71.4)	-	10 (45.5)	3 (13.6)	5 (35.7)	1 (7.1)	6 (75.0)	2 (25.0)	11 (40.7)	4 (14.8)	32 (50.0)	9 (14.1)
		I 8 (42.1)	1 (5.3)	10 (71.4)	4 (28.6)	4 (50.0)	1 (12.5)	5 (50.0)	2 (20.0)	2 (25.0)	1 (12.5)	25 (49.0)	8 (15.7)
Gastro-intestinal	Nausea	C 4 (57.1)	-	15 (68.2)	1 (4.5)	10 (71.4)	1 (7.1)	5 (62.5)	-	16 (59.3)	2 (7.4)	40 (62.5)	3 (4.7)
		I 7 (36.8)	1 (5.3)	6 (42.9)	–	3 (37.5)	-	5 (50.0)	–	4 (50.0)	1 (12.5)	22 (43.1)	2 (3.9)
	Vomiting	C 3 (42.9)	1 (14.3)	8 (36.4)	–	6 (42.9)	-	1 (12.5)	–	11 (40.7)	2 (7.4)	23 (35.9)	3 (4.7)
		I 2 (10.5)	1 (5.3)	2 (14.3)	–	2 (25.0)	-	1 (10.0)	–	4 (50.0)	1 (12.5)	9 (17.6)	2 (3.9)
	Diarrhea	C 4 (57.1)	1 (14.3)	4 (18.2)	–	1 (7.1)	-	1 (12.5)	–	8 (29.6)	–	17 (26.6)	1 (1.6)
		I 6 (31.6)	1 (5.3)	5 (35.7)	–	3 (37.5)	-	6 (60.0)	–	2 (25.0)	–	19 (37.3)	1 (2.0)
Other	Fatigue	C 6 (85.7)	1 (14.3)	8 (36.4)	–	3 (21.4)	-	3 (37.5)	1 (12.5)	8 (29.6)	3 (11.1)	25 (39.1)	5 (7.8)
		I 8 (42.1)	2 (10.5)	5 (35.7)	1 (7.1)	4 (50.0)	-	6 (60.0)	2 (20.0)	2 (25.0)	–	21 (41.2)	5 (9.8)

Abbreviations: C, Continuous azenosertib dosing; I, Intermittent azenosertib dosing; MTD, maximum tolerated dose; PLD, pegylated liposomal doxorubicin.
*All doses were at or below the MTD
**A MTD for Gemcitabine + Azenosertib has not been determined, further dose cohorts are ongoing.

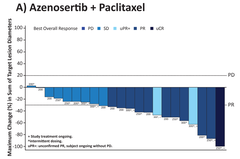
Table 3: Clinical Activity of Azenosertib Doublets

Endpoint	Azenosertib + Paclitaxel (N=26)	Azenosertib + Carboplatin (N=36)	Azenosertib + Gemcitabine (N=18)	Azenosertib + PLD (N=35)	Total (N=115)
Response-Evaluable (N)	22	28	13	31	94
ORR (confirmed), N (%)	11 (50.0)	10 (35.7)	5 (38.5)	6 (19.4)	32 (34.0)
Median DOR (95% CI) in months	5.6 (3.8-NE)	11.4 (8.3-NE)	6.2 (NE)	7.3 (1.5-NE)	8.3 (5.6-12.4)
Clinical Benefit Rate (CR + PR + SD for ≥ 16 weeks), N (%)	18 (81.8)	16 (57.1)	6 (46.2)	24 (77.4)	64 (68.1)
Median PFS (95% CI) in months	7.4 (5.5-NE)	10.4 (3.3-14.5)	8.3 (3.3-NE)	6.3 (3.7-11.0)	9.0 (5.8-13.7)

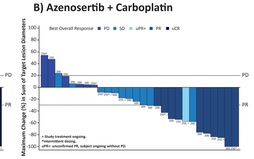
Response evaluable subjects are treated subjects with baseline measurable disease per RECIST version 1.1 and at least one post-baseline assessment. All objective responses were confirmed per RECIST v 1.1. Abbreviations: CR, complete response; DOR, duration of response; NE, non-evaluable; ORR, objective response rate; PFS, progression-free survival; PLD, pegylated liposomal doxorubicin; PR, partial response; SD, stable disease.

Figure 5: Waterfall Plots

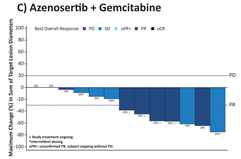
A) Azenosertib + Paclitaxel



B) Azenosertib + Carboplatin

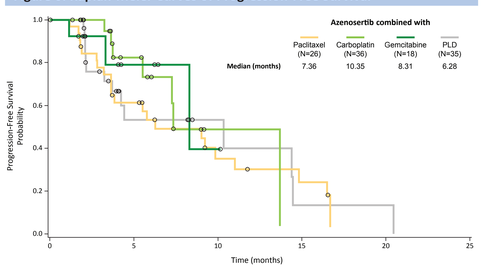


C) Azenosertib + Gemcitabine



Abbreviations: CR, complete response; NE, non-evaluable; PD, progressive disease; PR, partial response; SD, stable disease; uCR, unconfirmed complete response; uPR, unconfirmed partial response.

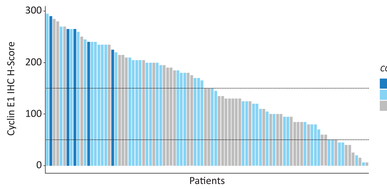
Figure 6: Kaplan-Meier Curves of Progression-Free Survival



Abbreviations: PLD, pegylated liposomal doxorubicin

Figure 7: Cyclin E1 Immunohistochemistry (IHC)

- Anti-Cyclin E1 mouse monoclonal antibody (Abcam CCNE1/2460)
- H-score > 50 includes all *CCNE1* amplified tumors
- Prevalence of Cyclin E1-IHC positive, H-score > 50 in this data set is 87%



H-Score*	> 150	≤ 150 to > 50	≤ 50
CCNE1 Amplified	5	0	0
CCNE1 Not Amplified	25	15	6
Tissue Not Evaluated for Amplification	16	21	6

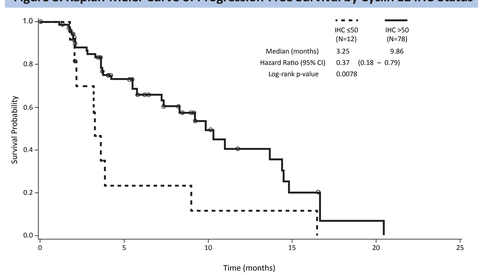
*H-scores calculated by using the percentage of cells (0 to 100%) with intensity of Cyclin E1 expression (0 to 3)

Table 4: Objective Responses by Cyclin E1 IHC-Status

Endpoints	Azenosertib + Paclitaxel	Azenosertib + Carboplatin	Azenosertib + Gemcitabine	Azenosertib + PLD	Total
Response Evaluable with IHC (N) *	19	22	13	28	82
Overall Response Rate, n (%)	10 (52.6)	8 (36.4)	5 (38.5)	6 (21.4)	29 (35.4)
Response Evaluable IHC H-Score >50 (N)	19	18	11	22	70
Overall Response Rate, n (%)	10 (52.6)	8 (44.4)	5 (45.5)	5 (22.7)	28 (40.0)
Response Evaluable IHC H-Score ≤50 (N)	0	4	2	6	12
Overall Response Rate, n (%)	NA	0	0	1 (16.7)	1 (8.3)

Response evaluable subjects are treated subjects with baseline measurable disease per RECIST version 1.1 and at least one post-baseline assessment. The ORRs for subjects with available IHC data appear to be consistent across cohorts with the overall population.Abbreviations: IHC, immunohistochemistry; PLD, pegylated liposomal doxorubicin.

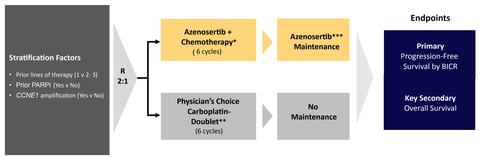
Figure 8: Kaplan-Meier Curve of Progression-Free Survival by Cyclin E1 IHC-Status



Abbreviations: CI, confidence interval; IHC, immunohistochemistry

Figure 9: Proposed Randomized Phase 3 Trial Design of Azenosertib + Chemotherapy in Cyclin E1+ Recurrent Platinum-Sensitive Ovarian Cancer

Key Eligibility: High-Grade Serous Ovarian Cancer; ECOG performance status 0-1; ≥1L Prior Line of Platinum-based chemotherapy; Platinum-Sensitive (Platinum-free interval ≥ 6 months); Prior Bevacizumab & PARPi if eligible and per regional standard of care; Cyclin E1+ (either *CCNE1* amplified and/or Cyclin E1 IHC-Positive)



*Paclitaxel or Carboplatin
**Paclitaxel or Pegylated Liposomal Doxorubicin
***Azenosertib, 400 mg QD 5:2
Abbreviations: 5:2, 5-days of treatment followed by 2-days off treatment; BICR, blinded independent central review; ECOG, Eastern Cooperative Oncology Group; PARPi, PARP-inhibitor.

CONCLUSIONS

- Azenosertib is active with chemotherapy and can be safely combined. RP2Ds are:
  - Paclitaxel 80 mg/m² on D1, D8, D15 (28-day cycles) + Azenosertib 300 mg QD 5:2
  - Carboplatin AUC 5 mg/mL*min on D1 (21-day cycles) + Azenosertib 200 mg QD 5:2
  - PLD 40 mg/m² D1 (28-day cycles) + Azenosertib 400 mg QD 5:2
  - Gemcitabine + Azenosertib has exciting and durable activity, a MTD has not been determined, further dose cohorts are ongoing
- Azenosertib-chemotherapy doublet combinations have a longer ORR, mDOR, and mPFS compared to historic control data for single-agent chemotherapy
- Cyclin E1 status predicts the benefit from the addition of azenosertib to single-agent chemotherapy suggesting that azenosertib restores chemotherapy sensitivity in heavily pre-treated platinum-resistant ovarian cancer
- Azenosertib + chemotherapy has a high level of clinical activity and safety, supporting a randomized study comparing this combination to carboplatin-doublet chemotherapy in platinum sensitive ovarian cancer

Abbreviations: 5:2, 5-days of treatment followed by 2-days off treatment; mDOR, median duration of response; mPFS, median progression-free survival; MTD, maximum tolerated dose; ORR, objective response rate; PLD, pegylated liposomal doxorubicin; RP2D, recommended phase 2 dose.

ACKNOWLEDGMENTS

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