TRILACICLIB IMPROVES OVERALL SURVIVAL WHEN GIVEN WITH GEMCITABINE/CARBOPLATIN IN PATIENTS WITH METASTATIC TRIPLE-NEGATIVE BREAST CANCER: FINAL ANALYSIS OF A RANDOMIZED PHASE 2 TRIAL

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INTRODUCTION

- Chemotherapy remains the mainstay of treatment for most patients with metastatic triple-negative breast cancer (mTNBC)[1]
- Chemotherapy-induced damage to hematopoietic stem and progenitor cells (HSPCs) can lead to depletion of lymphocyte populations, which may adversely affect the ability of the patient's immune system to mount an effective antitumor response[2,3]
- Trilaciclib is an intravenous CDK4/6 inhibitor that transiently arrests HSPCs and lymphocytes in the presence of chemotherapy to protect them from chemotherapy-induced damage[3]
  - Preclinically, the addition of trilaciclib to chemotherapy/immune checkpoint inhibitor regimens has been shown to enhance antitumor response and overall survival (OS) through modulation of the proliferation and composition of lymphocyte subsets in the tumor microenvironment and increased effector function[3]
- Preliminary data from a phase 2 trial showed that administering trilaciclib prior to gemcitabine plus carboplatin (GCb) significantly increased OS compared with GCb alone among patients with mTNBC[4]
- Here, we report final antitumor efficacy results for the whole study population, and in cohorts according to CDK4/6 dependence and immune subtyping, including levels of programmed death ligand-1 (PD-L1) expression

METHODS

Study Design

- This was a randomized, open-label, phase 2 study of patients with mTNBC who had received ≤2 previous lines of chemotherapy in the recurrent/metastatic setting (NCT02978716)[4]
- Patients were randomized (1:1:1) to receive GCb on days 1 and 8 (group 1), trilaciclib prior to GCb on days 1 and 8 (group 2), or trilaciclib alone on days 1 and 8 and prior to GCb on days 2 and 9 (group 3), in 21-day cycles
- Progression-free survival (PFS) and OS (prespecified secondary endpoints) were assessed in the intention-to-treat population, and objective response rate (ORR) in response-evaluable patients
- To assess the effect of trilaciclib on the composition of lymphocyte subsets and clonal expansion, T-cell receptor (TCR) β CDR3 regions were amplified and sequenced from purified genomic DNA in peripheral blood mononuclear cells isolated from whole blood samples collected on day 1 of cycles 1 (baseline), 3, and 5

Cohort Analysis

- RNA was isolated from archival tumor tissue collected at screening
- Patient tumors were retrospectively characterized as CDK4/6 dependent, independent, or of variable/indeterminate dependence according to the established PAM50 and Lehmann TNBCtype-4 signatures[5–7] (**Table 1**)

Table 1. Characterization of CDK4/6 Dependency According to Signature

Signature	Known Dependence	Known Independence	Variable/Indeterminate Dependence
PAM50	–	Basal-like	HER2-enriched, normal-like, luminal A/B
Lehmann TNBCtype-4	LAR	–	Basal-like 1/2, mesenchymal

HER2, human epidermal growth factor receptor 2; LAR, luminal androgen receptor; TNBC, triple-negative breast cancer.

- PD-L1 expression was scored as negative or positive if < 1% or ≥ 1% of the total tumor area contained PD-L1–labelled immune cells, respectively, using the Ventana SP142 assay
- Three RNA-based immune signatures were identified via literature review:
  - An interferon-gamma signature (IFNγ) based on 6 genes[8] and an expanded IFNγ signature based on 18 genes[8]
    - Patients were classified as having high or low gene expression
  - An immune signature based on 6 identified immune response subtypes[9]
    - Patients were classified as being IFNγ dominant (Class 2) or not
- Association of CDK4/6 dependence, PD-L1 expression, and immune signatures with antitumor efficacy was assessed using proportional hazards regression

RESULTS

Patients

- A total of 102 eligible patients were randomly assigned to group 1 (n = 34), group 2 (n = 33), or group 3 (n = 35)
- Median (range) follow-up was 8.4 (0.1–25.7) months for group 1, 14.0 (1.3–33.6) months for group 2, and 15.3 (3.5–33.7) months for group 3
- As previously described, baseline characteristics were similar between treatment groups[4]

Antitumor Efficacy in the Overall Population

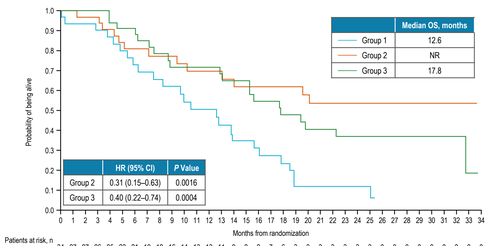
- Patients receiving trilaciclib prior to GCb had higher ORR, longer PFS, and significantly improved OS compared with patients receiving GCb alone (**Table 2; Figure 1**)

Table 2. Outcomes in the Overall Population: Tumor Response, PFS, and OS

	Group 1	Group 2	Group 3	Groups 2 and 3
Patients, n	34	33	35	68
ORR,[a] n/N (%)	7/24 (29.2)	15/30 (50.0)	12/31 (38.7)	27/61 (44.3)
Median PFS,[a] months (95% CI)	5.7 (3.3–9.9)	9.4 (6.1–11.9)	7.3 (6.2–13.9)	9.0 (6.4–11.3)
P value	–	0.2099	0.1816	0.1291
HR (95% CI)	–	0.62 (0.32–1.20)	0.63 (0.32–1.22)	0.62 (0.36–1.10)
Median OS,[b] months (95% CI)	12.6 (6.3–15.6)	NR (10.2–NR)	17.8 (12.9–32.7)	19.8 (14.0–NR)
P value	–	0.0016	0.0004	< 0.0001
HR (95% CI)	–	0.31 (0.15–0.63)	0.40 (0.22–0.74)	0.37 (0.21–0.63)

Group 1: chemotherapy on days 1 and 8; group 2: trilaciclib and chemotherapy on days 1 and 8; group 3: trilaciclib alone on days 1 and 8 and with chemotherapy on days 2 and 9. HR and *P* values are for comparisons between group 2 and group 1, group 3 and group 1, and between groups 2 and 3 combined and group 1.
[a] ORR/PFS data are from 15 May, 2020 data cutoff.
[b] OS data are from final database lock, with data cutoff of 17 July, 2020.
HR, hazard ratio; NR, not reached; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

Figure 1. OS



HR and *P* values are for comparisons between group 2 and group 1, and group 3 and group 1.
HR, hazard ratio; NR, not reached.

Outcomes According To CDK4/6 Subtyping

- ORR, PFS, and OS were similar in tumors categorized as CDK4/6 dependent, independent, or indeterminate
  - Trilaciclib did not impair the efficacy of GCb in patients with known CDK4/6-dependent tumors (luminal androgen receptor according to the Lehmann signature) or CDK4/6-variable tumors (non-basal-like according to the PAM50 signature) (**Table 3**)
- Outcomes were similar in patients with known CDK4/6-independent (basal-like according to the PAM50 signature) or CDK4/6-variable tumors (basal-like 1/2 or mesenchymal according to the Lehmann signature) (data not shown)

Table 3. Efficacy Among Patients with Known CDK4/6-Dependence or Variable/Indeterminate Dependency

	Group 1	Group 2	Group 3	Groups 2 and 3
Lehmann signature (LAR), n	9	10	9	19
ORR, n (%)	2 (22.2)	4 (40.0)	1 (11.1)	5 (26.3)
Median PFS, months (95% CI)	8.3 (4.8–NR)	11.6 (9.4–NR)	5.9 (2.7–NR)	9.4 (6.5–NR)
P value	–	0.1336	0.6376	0.4188
HR (95% CI)	–	0.39 (0.1–1.4)	1.3 (0.4–4.7)	0.65 (0.2–1.8)
Median OS, months (95% CI)	9.7 (7.5–NR)	NR (9.4–NR)	15.3 (7.5–NR)	15.3 (9.4–NR)
P value	–	0.0052	0.1397	0.008
HR (95% CI)	–	0.18 (0.0–0.7)	0.49 (0.2–1.3)	0.32 (0.1–0.8)
PAM50 signature (non-basal), n	12	10	14	24
ORR, n (%)	4 (33.3)	4 (40.0)	5 (35.7)	9 (37.5)
Median PFS, months (95% CI)	8.3 (4.8–NR)	11.9 (8.8–NR)	7.3 (5.9–NR)	9.4 (7.3–NR)
P value	–	0.1255	0.4794	0.191
HR (95% CI)	–	0.42 (0.1–1.3)	0.71 (0.3–1.9)	0.57 (0.2–1.3)
Median OS, months (95% CI)	10.1 (7.5–18.8)	NR (9.4–NR)	22.3 (13.1–NR)	22.3 (13.0–NR)
P value	–	0.0164	0.0095	0.003
HR (95% CI)	–	0.30 (0.1–0.8)	0.32 (0.1–0.8)	0.33 (0.2–0.7)

Group 1: chemotherapy on days 1 and 8; group 2: trilaciclib and chemotherapy on days 1 and 8; group 3: trilaciclib alone on days 1 and 8 and with chemotherapy on days 2 and 9. HR and *P* values are for comparisons between group 2 and group 1, group 3 and group 1, and between groups 2 and 3 combined and group 1.
HR, hazard ratio; LAR, luminal androgen receptor; NR, not reached; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

Outcomes According To Immune Subtyping

- Expression of PD-L1 was considered positive in 49 of 85 (57.6%) tumor tissue samples, including 32 of 58 (55.2%) in the trilaciclib groups and 17 of 27 (63.0%) in the GCb group
- Administering trilaciclib prior to GCb enhanced OS irrespective of PD-L1 status but with a larger OS benefit in the PD-L1–positive population (**Table 4**)

Table 4. Tumor Response, PFS, and OS According to PD-L1 Status

	PD-L1 Positive				PD-L1 Negative			
	Group 1	Group 2	Group 3	Groups 2 and 3	Group 1	Group 2	Group 3	Groups 2 and 3
Patients analyzed, n	17	16	16	32	10	10	16	26
ORR, n (%)	4 (23.5)	8 (50.0)	7 (43.8)	15 (46.9)	3 (30.0)	4 (40.0)	4 (25.0)	8 (30.8)
Median PFS, months (95% CI)	5.4 (3.3–NR)	7.9 (6.1–NR)	10.9 (6.2–NR)	9.7 (6.2–15.5)	9.2 (8.3–NR)	11.9 (8.8–NR)	9.0 (6.4–NR)	9.4 (6.5–14.6)
P value	–	0.492	0.075	0.149	–	0.376	0.488	0.943
HR (95% CI)	–	0.74 (0.3–1.7)	0.41 (0.2–1.1)	0.57 (0.3–1.2)	–	0.60 (0.2–1.9)	1.47 (0.5–4.3)	0.97 (0.4–2.5)
Median OS, months (95% CI)	10.5 (6.3–18.8)	20.1 (10.2–NR)	32.7 (15.3–NR)	32.7 (17.7–NR)	13.9 (12.6–NR)	NR (9.4–NR)	17.8 (12.9–NR)	17.8 (13.1–NR)
P value	–	0.037	0.01	0.004	–	0.077	0.198	0.093
HR (95% CI)	–	0.38 (0.2–1.0)	0.30 (0.1–0.8)	0.34 (0.2–0.7)	–	0.35 (0.1–1.2)	0.55 (0.2–1.4)	0.48 (0.2–1.2)

Group 1: chemotherapy on days 1 and 8; group 2: trilaciclib and chemotherapy on days 1 and 8; group 3: trilaciclib alone on days 1 and 8 and with chemotherapy on days 2 and 9. HR and *P* values are for comparisons between group 2 and group 1, group 3 and group 1, and between groups 2 and 3 combined and group 1.
HR, hazard ratio; NR, not reached; ORR, objective response rate; OS, overall survival; PD-L1, programmed death ligand-1; PFS, progression-free survival.

- Administering trilaciclib prior to GCb enhanced PFS and OS irrespective of immune status, with a similar OS benefit between patients with high or low immune-related gene expression (**Table 5**)

Table 5. Tumor Response, PFS, and OS According to Immune Subtypes

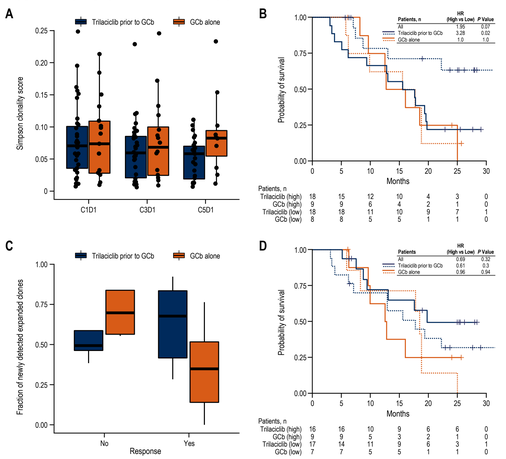
	High/Class 2				Low/Not Class 2			
Subtype	Group 1	Group 2	Group 3	Groups 2 and 3	Group 1	Group 2	Group 3	Groups 2 and 3
IFNγ signature,[8] n	13	11	12	23	9	15	15	30
ORR, n (%)	5 (38.5)	7 (63.6)	6 (50.0)	13 (56.5)	2 (22.2)	5 (33.3)	6 (40.0)	11 (36.7)
Median PFS, months (95% CI)	5.7 (5.4–NR)	13.0 (11.3–NR)	9.0 (6.5–NR)	11.3 (7.3–NR)	8.3 (2.0–NR)	13.9 (3.9–NR)	7.9 (6.1–NR)	8.8 (6.1–14.6)
P value	–	0.0931	0.2797	0.0871	–	0.7513	0.846	0.7545
HR (95% CI)	–	0.40 (0.1–1.2)	0.59 (0.2–1.5)	0.49 (0.2–1.1)	–	0.85 (0.3–2.3)	0.90 (0.3–2.7)	0.87 (0.3–2.2)
Median OS, months (95% CI)	12.8 (9.7–NR)	20.1 (7.1–NR)	22.3 (17.8–NR)	22.3 (15.3–NR)	8.3 (6.3–NR)	15.3 (8.7–NR)	19.6 (10.2–NR)	15.6 (12.9–NR)
P value	–	0.0906	0.0257	0.0152	–	0.0207	0.0553	0.0168
HR (95% CI)	–	0.44 (0.2–1.2)	0.35 (0.1–0.9)	0.40 (0.2–0.9)	–	0.30 (0.1–0.9)	0.41 (0.2–1.1)	0.37 (0.2–0.9)
Expanded IFNγ signature,[9] n	13	10	14	24	9	16	13	29
ORR, n (%)	5 (38.5)	6 (60.0)	6 (42.9)	12 (50.0)	2 (22.2)	5 (38.5)	7 (43.8)	12 (41.4)
Median PFS, months (95% CI)	5.7 (4.8–NR)	11.3 (8.8–NR)	9.0 (6.2–NR)	9.7 (7.3–20.1)	8.3 (2.0–NR)	13.9 (5.9–NR)	7.9 (6.1–NR)	9.4 (6.1–15.5)
P value	–	0.0924	0.2336	0.0765	–	0.9265	0.7972	0.8653
HR (95% CI)	–	0.39 (0.1–1.2)	0.56 (0.2–1.5)	0.47 (0.2–1.1)	–	1.0 (0.4–2.7)	1.2 (0.4–3.6)	1.1 (0.4–2.7)
Median OS, months (95% CI)	12.8 (9.7–NR)	NR (7.1–NR)	19.8 (15.3–NR)	20.1 (15.3–NR)	9.1 (6.3–NR)	17.7 (12.9–NR)	14.0 (10.2–NR)	15.6 (12.9–NR)
P value	–	0.0428	0.0692	0.0185	–	0.0643	0.0364	0.0226
HR (95% CI)	–	0.38 (0.1–1.0)	0.44 (0.2–1.1)	0.41 (0.2–0.9)	–	0.40 (0.1–1.1)	0.38 (0.1–1.0)	0.40 (0.2–0.9)
Six-class immune signature,[9] n	10	17	18	35	12	9	9	18
ORR, n (%)	3 (30.0)	9 (52.9)	8 (44.4)	17 (48.6)	4 (33.3)	3 (33.3)	4 (44.4)	7 (38.9)
Median PFS, months (95% CI)	9.2 (5.4–NR)	8.8 (6.2–NR)	10.9 (6.5–NR)	10.9 (6.5–14.0)	5.4 (3.3–NR)	7.3 (1.2–NR)	9.7 (2.1–NR)	9.4 (5.9–15.6)
P value	–	0.5685	0.3952	0.4029	–	0.3799	0.9662	0.5126
HR (95% CI)	–	0.75 (0.3–2.0)	0.65 (0.2–1.8)	0.69 (0.3–1.7)	–	0.63 (0.2–1.8)	0.99 (0.4–2.7)	0.76 (0.3–1.8)
Median OS, months (95% CI)	12.8 (5.8–NR)	NR (13.0–NR)	22.3 (15.3–NR)	32.7 (15.3–NR)	10.2 (7.5–18.8)	13.1 (8.7–NR)	14.8 (9.4–NR)	13.1 (9.4–NR)
P value	–	0.1177	0.0822	0.0539	–	0.0971	0.1376	0.0609
HR (95% CI)	–	0.47 (0.2–1.2)	0.45 (0.2–1.1)	0.46 (0.2–1.0)	–	0.42 (0.1–1.2)	0.52 (0.2–1.3)	0.49 (0.2–1.0)

Group 1: chemotherapy on days 1 and 8; group 2: trilaciclib and chemotherapy on days 1 and 8; group 3: trilaciclib alone on days 1 and 8 and with chemotherapy on days 2 and 9. HR and *P* values are for comparisons between group 2 and group 1, group 3 and group 1, and between groups 2 and 3 combined and group 1.
Class 2 was defined as IFN-γ dominant.
Not adjusted for multiplicity.
HR, hazard ratio; IFNγ, interferon-gamma signature; NR, not reached; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

TCR Analysis

- There was a significant decrease in Simpson clonality among patients who received trilaciclib prior to GCb compared with GCb alone ($P_{INTERACTION}$ = 0.012; **Figure 2A**)
- When patients were stratified above or below median Simpson clonality, there was a trend for improved OS among patients with decreased peripheral clonality, with a statistically significant improvement among patients receiving trilaciclib (*P* = 0.02) (**Figure 2B**)
- Responders receiving trilaciclib in groups 2 and 3 had more newly detected expanded clones compared with responders receiving GCb alone (*P* = 0.09), with no difference between responders and nonresponders in the trilaciclib groups (*P* = 0.79; **Figure 2C**)
- Although not statistically significant, when patients were stratified above or below median fraction of newly detected expanded clones, OS was improved among patients with a higher fraction of newly detected expanded clones who received trilaciclib (**Figure 2D**)

Figure 2. TCR Clonality and Expansion



Figures 2A and **2C** show median values with 25% and 75% quartiles. For Kaplan–Meier estimates of probability of survival, patients were stratified by high (equal or above median; solid lines) and low (below median; dashed lines) Simpson clonality score (**Figure 2B**) and fraction of newly detected expanded clones (**Figure 2D**). HR indicates ratio of high relative to low. Values were calculated using Cox proportional hazards regression and the Wald test to determine statistical significance.
C, cycle; D, day; GCb, gemcitabine and carboplatin; HR, hazard ratio.

CONCLUSIONS

- Mature data from this study were consistent with the primary analysis,[4] confirming that administering trilaciclib prior to GCb enhances antitumor efficacy compared with administering GCb alone, with statistically significant improvements in OS
- Subgroup analyses suggest that administering trilaciclib prior to GCb benefits patients regardless of CDK4/6 dependence status and PD-L1 expression
- Data from immune subtyping analyses and TCR immunosequencing suggest that administering trilaciclib prior to GCb both preserved and enhanced immune system function
- Further investigation into the association between enhanced antitumor immunity and improved OS is warranted among patients receiving trilaciclib and chemotherapy for the treatment of mTNBC

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