Supplementary Appendix for "IOLite: Phase 1b Trial of Doublet/Triplet Combinations of Dostarlimab With Niraparib, Carboplatin-Paclitaxel, With or Without Bevacizumab in Patients With Advanced Cancer"

Table of Contents

Dose escalation

In parts A, C, and D, a cohort of 6 patients was initially enrolled to receive combination treatment. Each combination was considered safe if all patients who were evaluable for safety completed the combination treatment and ≤1 of 6 patients experienced a dose-limiting toxicity (DLT). DLTs were defined as follows: any treatment-related grade 4 nonhematologic clinical adverse event (AE); any treatment-related grade 3 nonhematologic clinical AE lasting more than 3 days; any treatment-related grade 3 or 4 nonhematologic laboratory abnormality that leads to hospitalization or persists for 7 days or more; grade 4 thrombocytopenia that persists for 7 days or more or grade 3 or 4 thrombocytopenia associated with clinically significant bleeding; grade 4 neutropenia, grade 3 or 4 neutropenia associated with infection, grade 3 or 4 febrile neutropenia that persists for days or more; grade 4 anemia or grade 3 anemia requiring blood transfusion; any treatment-related toxicity leading to at least a 2-week delay in initiating cycle 2; any treatment-related grade 5 AE. If 2 of 6 patients experienced DLTs, 6 additional patients were enrolled. If ≤3 of 12 patients experienced DLTs, the combination dosage was considered safe. If ≥3 of 6 or ≥4 of 12 patients experienced DLTs in part A or C, then the combination dosage was considered to have exceeded the maximum tolerated dose, and the recommended phase 2 dose (RP2D) was the prior dose level. Part B enrolled 12 patients initially, and the combination treatment was considered safe if ≤3 of 12 patients experienced DLTs. In parts A and C, once niraparib 200 mg once daily (QD) was considered safe, 6 patients were enrolled at 300 mg QD and were subject to the safety determination described previously. For all parts, the RP2D for combination treatments was confirmed by the investigators based on multiple endpoints: the DLT rate of combination treatment, rate of dose modifications for non-DLT AEs, ability to

manage toxicities, pharmacokinetics (PK), and signs of clinical efficacy. In parts A and C, we identified the highest dose of niraparib that was safely combined with the recommended dose and regimen of dostarlimab or dostarlimab and bevacizumab. In parts B and D, we confirmed the recommended dose and regimen of dostarlimab combination with carboplatin-paclitaxel, with or without bevacizumab.

Receptor occupancy

Receptor occupancy (RO) assessments are used routinely as pharmacodynamic measures to confirm target engagement during preclinical and clinical development of biopharmaceuticals such as dostarlimab. Although target engagement of dostarlimab on PD-1 receptors has been previously studied, RO in the presence of drug combinations has not been established. The RO assay used in this trial provides a measure of dostarlimab binding to PD-1 receptors on cluster of differentiation (CD)3+ T-cells from patients treated in combination with niraparib (part A), carboplatin/paclitaxel (part B), and bevacizumab (part D). Whole blood samples collected from a subset of patients from parts A, B, and D at screening, before cycle 1 day 1 (C1D1) and C2D2 doses, and at end of treatment (EOT) were assayed for RO by flow cytometry as described in the Methods of the primary manuscript. %RO was calculated as the percentage of the ratio of PD-1 positive cells in the isotype-saturated condition and the dostarlimab-saturated condition in CD3+ T-cells. Based on our analysis, following a single dostarlimab dose (500 mg), the mean PD-1 RO was 97.45% prior to the second scheduled dose of study drug (before C2D2 dose, **eFigure 1**). The overall mean PD-1 RO was 99.01% by end of treatment. This result is consistent with RO data previously published for dostarlimab monotherapy.[1] These data demonstrate that the target occupancy of dostarlimab is not impacted in the presence of different drug combinations.

RO methodology

The RO assay was previously developed by Tesaro and performed at a third-party specialty vendor lab (PrimityBio, CA). A sample of 8.0 mL of whole blood was collected at screening, before C1D1 and C2D2 doses, and at EOT in CPT tubes with Na-heparin from patients (n = 27) enrolled in parts A, B, and D of the study. The blood samples collected were centrifuged, and peripheral blood mononuclear cells (PBMCs) were separated at the clinical site prior to shipping to Primity Bio at ambient temperature within a 24-h window. PBMCs were incubated in vitro with a saturating concentration of either unconjugated dostarlimab (20 µg/mL) or the corresponding dostarlimab isotype control (IgG4 antibody; 20 µg/mL). Whereas the isotype saturated samples reveal the amount of dostarlimab that was bound in vivo ("bound receptor"), the dostarlimab-saturated sample reveals the amount of PD-1 receptors on the cell surface ("total receptors"). The PBMCs were washed twice and stained for flow cytometric analysis using a qualified phenotyping antibody panel that included the following markers: Viability Dye, anti-CD3 antibody, anti-CD45 antibody, and anti-IgG4 secondary antibody (detects both dostarlimab and isotype control). Cells were then washed and fixed before data acquisition using LSRII flow cytometers. The acquired data were analyzed by gating methods to identify specific cell subsets. %RO was calculated as the percentage of the ratio of PD-1 positive cells in the isotype-saturated condition and the dostarlimab-saturated condition ("bound/total" receptors) in CD3+T-cells.

eTable 1. First Dose of Dostarlimab 500 mg Q3W PK Summary

IOLIte Trial	C_{max}, µg/mL	C_{last}, µg/mL	t_{max}, h	AUC_{last}, µg*h/mL
Part A				
200 mg of niraparib QD (n = 16)	159 ± 27.7	40.3 ± 16.5	0.817 (0.50-2.58)	26800 ± 9810
300 mg of niraparib QD (n = 6)	139 ± 37.9	30.3 ± 8.16	1.750 (0.50-23.17)	29400 ± 9260
Part B (n = 14)	148 ± 31.3	32.8 ± 8.10	1.000 (0.55-27.28)	28100 ± 8440
Part C				
200 mg of niraparib QD (n = 6)	139 ± 24.1	43.0 ± 16.6	1.200 (0.50-4.48)	29800 ± 8140
300 mg of niraparib QD (n = 7)	158 ± 48.4	37.9 ± 14.4	0.583 (0.50-2.35)	26300 ± 4420
Part D (n = 6)	189 ± 36.0	39.0 ± 14.5	1.250 (0.50-25.17)	32800 ± 9180
GARNET Trial (n = 6)	174 ± 35.02	40.2 ± 9.31	0.960 (0.50-3.02)	36300 ± 6800

Abbreviations: AUC_{last}, area under the concentration–time curve from 0 to last measurable plasma concentration; C_{last}, the last measurable concentration of first dose; C_{max}, maximum plasma concentration; PK, pharmacokinetics; Q3W, every 3 weeks; t_{max}, time to maximum serum concentration.
Data are reported as ± standard deviation of the mean or median (range) for t_{max}.

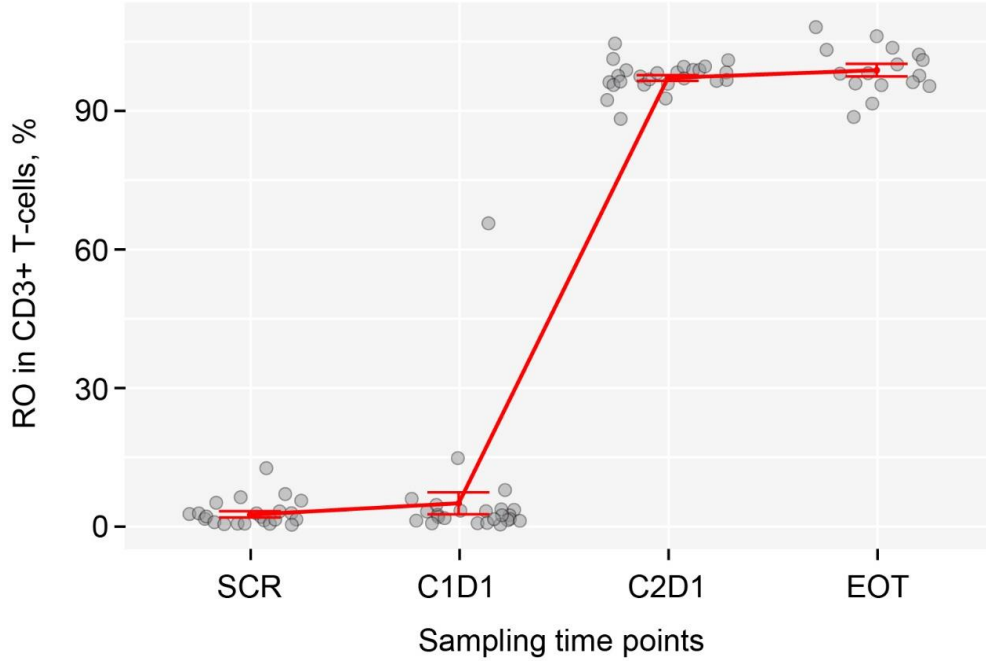
eTable 2. First Dose of Niraparib 200 mg or 300 mg QD PK Summary

IOLite Trial	C_{max}, µg/mL	C_{last}, µg/mL	t_{max}, h	AUC_{last}, µg*h/mL
Part A				
200 mg of niraparib QD (n = 15)	0.461 ± 0.240	0.192 ± 0.131	2.250 (1.75-7.02)	6.04 ± 3.43
300 mg of niraparib QD (n = 6)	0.626 ± 0.455	0.342 ± 0.330	4.083 (2.02-23.17)	7.83 ± 4.76
Part C				
200 mg of niraparib QD (n = 6)	0.244 ± 0.089	0.161 ± 0.107	3.975 (2.00-23.33)	3.45 ± 1.23
300 mg of niraparib QD (n = 7)	0.892 ± 0.464	0.479 ± 0.420	4.050 (2.05-22.60)	11.4 ± 5.54

Abbreviations: AUC_{last}, area under the concentration–time curve from 0 to last measurable concentration; C_{last}, last measurable plasma concentration after first dose; C_{max}, maximum plasma concentration; PK, pharmacokinetic; QD, once daily; t_{max}, time to maximum plasma concentration.

Data are reported as ± standard deviation of the mean or median (range).

eFigure 1. PD-1 Receptor Occupancy by Dostarlimab on CD3+ T-Cells From Whole Blood Samples Collected From Patients on Study



Flow cytometry-based PD-1 RO analysis was performed on whole blood samples collected from patients (n = 27) enrolled in parts A, B, and D of the study as described in the Methods section. %RO was plotted against the sampling time points. All results reported on the RO analysis set were from patients who have received dostarlimab and have at least 1 predose and 1 on treatment pharmacodynamic sample available. Data were pooled across study sites. Descriptive statistics were used to summarize the percent PD-1 RO in CD3+ T-cells at each time point collected. A single dose of dostarlimab was sufficient to occupy PD-1 receptors significantly, $P < 2.2e{-}16$. C indicates cycle; D, day; EOT, end of treatment; RO, receptor occupancy; SCR, screening.

Supplemental appendix references

1. Oaknin A, Tinker AV, Gilbert L, et al. Clinical activity and safety of the anti-programmed death 1 monoclonal antibody dostarlimab for patients with recurrent or advanced mismatch repair-deficient endometrial cancer: a nonrandomized phase 1 clinical trial. *JAMA Oncol.* 2020;6(11):1766-1772.