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Phase II study of rucaparib and nivolumab in patients with leiomyosarcoma

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ABSTRACT

Background Objective responses to immune checkpoint inhibitors (ICI) in leiomyosarcoma (LMS) are rare. Response rates may be increased by combination with other drugs known to promote immune infiltration, such as poly(ADP-ribose) polymerase (PARP) inhibitors, which have led to benefit in *BRCA*-altered uterine LMS. We therefore evaluated the combination of a PARP inhibitor, rucaparib, and the anti-programmed death receptor-1 monoclonal antibody, nivolumab, in patients with advanced LMS and investigated its effects on the tumor immune microenvironment.

Methods This was an open-label, single-center, single-arm, phase II study in patients with advanced refractory LMS. Full protocol available Patients were treated with rucaparib 600 mg orally, two times daily, continuously and nivolumab 480 mg intravenously on day 1 of a 28-day cycle. Re-staging scans were performed every 8 weeks. Blood and tissue samples were collected at baseline and at week 8 on treatment. The primary objective was the best objective response rate by 24 weeks using Response Evaluation Criteria in Solid Tumour (RECIST V.1.1). Secondary objectives included treatment-related toxicity, progression-free survival, overall survival, and changes in immune pathways in blood and tumor.

Results 20 patients with LMS were enrolled. There was one partial response (PR) (5%) in a patient with uterine LMS and a somatic *BRCA* deep deletion. 19 (95%) patients had a treatment-related adverse event (TRAE) and 7 (35%) had a grade 3 or higher TRAE. Interferon (IFN) α and γ hallmark pathways were more highly expressed in patients who derived benefit from treatment (at least stable disease by 16 weeks) vs those who did not in both baseline (adjusted p=0.005 for IFN-α, 0.03 for IFN-γ) and on-treatment biopsies (adjusted p=0.0002 for IFN-α, 0.0001 for IFN-γ), but the abundance of tumor immune cell populations did not differ between these groups at either time point.

Conclusion The addition of a PARP inhibitor did not improve the efficacy of ICI in LMS. Adverse events, especially due to overlapping toxicities, were frequent and often led to dose delays and modifications.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Objective responses to immune checkpoint inhibitors (ICI) in leiomyosarcoma (LMS) are rare. Poly(ADP-ribose) polymerase (PARP) inhibitors have led to benefit in *BRCA*-altered uterine LMS and promote tumor immune infiltration.

WHAT THIS STUDY ADDS

⇒ The combination of rucaparib and nivolumab led to a 5% response rate among 20 patients with LMS, which is not an improvement over ICI alone, and the only response was in a single patient with a deep *BRCA2* deletion. Adverse events were frequent.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Further evaluation of PARP inhibitors with ICI in LMS is not warranted.

BACKGROUND

Leiomyosarcoma (LMS), a tumor of smooth muscle origin, is one of the most common subtypes of soft tissue sarcoma.[1] Chemotherapy remains the standard of care for patients with metastatic disease. High expression of genes related to antigen presentation and T-cell infiltration has been noted in LMS tumors, similar to sarcomas such as undifferentiated pleomorphic sarcoma, where immune checkpoint inhibition is active.[2] Perplexingly, single-agent inhibitors of the immune checkpoint programmed death receptor-1 (PD-1) have demonstrated little activity in LMS,[3 4] possibly due to a preponderance of immunosuppressive tumor-associated macrophages in the tumor microenvironment.[5]

The efficacy of immune checkpoint inhibitors may be increased by combination with agents that enhance the antitumor immune response. One such class of drug with activity in LMS is inhibitors of poly(ADP-ribose)

polymerase (PARP)-1, which contributes to single-strand and double-strand DNA repair.[6] The development of PARP inhibitors has largely focused on homologous repair (HR)-deficient cancers such as *BRCA1/2*-altered breast and ovarian cancer. Recurrent alterations in HR genes have been identified in LMS tumors[7 8] and clinical benefit to PARP inhibition has been noted in patients with *BRCA2*-altered uterine LMS.[9]

PARP inhibitors have been shown to increase the activity of the type I (α) and type II (γ) interferon (IFN) pathways, activate the cGAS/STING pathway, increase T-cell infiltration in tumors, and upregulate programmed death ligand 1 (PD-L1) expression.[10–12] In earlier non-randomized studies of breast and ovarian cancer, the combination of a PARP inhibitor and immune checkpoint inhibition demonstrated promising antitumor activity, independent of *BRCA1/2* or HR deficiency status.[13–15]

Based on the hypothesis that PARP inhibition would modulate the tumor immune microenvironment, this study evaluated the combination of the PARP inhibitor rucaparib (Food and Drug Administration (FDA)-approved for *BRCA*-mutated, metastatic, castration-resistant prostate cancer and for maintenance in recurrent, *BRCA*-mutated ovarian cancer) and the anti-PD-1 monoclonal antibody nivolumab (approved for melanoma, urothelial, and renal cancers, among others).

METHODS

Patients

Patients 18 years or older with unresectable or metastatic LMS that was measurable by Response Evaluation Criteria in Solid Tumour (RECIST) V.1.1 were eligible. Patients were required to have had one to three prior lines of therapy. Additional inclusion criteria were adequate organ and bone marrow function and Eastern Cooperative Oncology Group performance status ≤2. No prior treatment with PARP inhibitors or immune checkpoint inhibitors was allowed. Patients with central nervous system disease <4 weeks from completion of prior therapy, or clinically significant, uncontrolled intercurrent illness including cardiac disease, symptomatic autoimmune disease, pneumonitis, or chronic steroid use were also excluded.

Study design and treatment

This was an open-label, single-center, single-arm, phase II study (online supplemental file 5). Rucaparib was administered at a starting dose of 600 mg orally, two times daily, continuously for 28 days. Nivolumab was administered at 480 mg intravenously on day 1 of every 4-week cycle. Patients underwent restaging scans every 8 weeks. Treatment was continued until disease progression, unacceptable toxicity, or patient withdrawal. Dose modification or interruption of rucaparib and dose delays of both drugs were permitted in case of toxicity. Liver function abnormalities were considered an overlapping toxicity of both drugs. Nivolumab was held for ≥grade 2 liver enzyme elevation. Rucaparib was continued with close monitoring for resolution upon ≤grade 3 elevations in alanine aminotransferase/aspartate aminotransferase (without accompanying rise in bilirubin) within 15 days of starting because such events are known to spontaneously improve if due to rucaparib alone. Any liver enzyme elevation >8 times normal required hold of both drugs, initiation of steroids, and close monitoring. Grade 3 or higher anemia required rucaparib to be held. Close monitoring was advised for persistent anemia, and in some cases, a hematology consult was necessary to rule out a myelodysplastic syndrome.

Endpoints and assessments

The primary objective of this study was to evaluate efficacy, as assessed by the best objective response rate (ORR) (complete or partial response (PR))by 24 weeks by RECIST V.1.1. Secondary endpoints included treatment-related toxicity, progression-free survival (PFS) and overall survival (OS). Safety was assessed at screening, continuously during treatment, and for 30 days after the last dose of study drug. Adverse events were assessed according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) V.5.0.

All patients who received ≥1 cycle of treatment with both nivolumab and rucaparib were considered evaluable for efficacy. Patients who missed an assessment for response due to clinical progression or treatment-related toxicities were considered non-responders for that time point. Patients were evaluable for safety if they received ≥1 dose of rucaparib and nivolumab.

Biomarker assessments

Patients were required to submit archival tissue for biomarker testing. On-treatment biopsies were conducted at week 8 when deemed safe and feasible by the treating physician and were optional at the time of progression. Research blood samples for circulating tumor DNA (ctDNA), cytokine, and T-cell flow cytometry analysis were collected at baseline, weeks 5, 9, 17, and 25 on treatment, and at end of treatment.

Tumor mutational profiling

DNA extracted from formalin-fixed paraffin-embedded (FFPE) archival tissue and matched normal blood were analyzed using the FDA-authorized next generation sequencing assay MSK-IMPACT. Total and allele-specific copy numbers were estimated from MSK-IMPACT data using the FACETS algorithm (V.0.5.6).[16] Sequencing data was analyzed using validated bioinformatics methods.[17 18]

Any somatic or germline nonsense, frameshift, or splice site mutation predicted to lead to loss of function of the encoded protein or homozygous deletion of a DNA damage repair (DDR) gene was considered deleterious.[19] In addition, missense mutations annotated as oncogenic by OncoKB were considered deleterious.[20] For each sample, tumor mutational burden (TMB) was calculated by dividing the total number of non-synonymous

mutations by the total number of sequenced base pairs. Fraction of the genome altered (FGA) was calculated as the sum of the absolute $\log_2$ copy number deviations exceeding 0.2, divided by the size of the analyzed genomic region. Microsatellite instability (MSI) status was assessed using MSIsensor.[21] Evidence of microsatellite instability at ≥10% of analyzable loci was considered MSI-high and mismatch repair deficient.[22]

Germline variant analysis and pathogenicity evaluation

At the time of data freeze, 15 patients had consented to analysis of germline variants via an Institutional Review Board (IRB) protocol (#12–245, part C; NCT01775072). Germline annotation for pathogenic or likely pathogenic variants was performed by MSK-IMPACT using a clinically validated platform.[18 23]

Cytokines and flow cytometry

Human peripheral blood mononuclear cells (PBMC) were separated from whole blood collected in sodium heparin CPT tubes (BD) by standard Ficoll density gradient centrifugation. Plasma supernatants from spun blood samples were collected and frozen at −80°C in 1.5 mL aliquots. Isolated PBMCs were washed several times to minimize platelets, counted, and cryopreserved in liquid nitrogen at between 5 million and 10 million cells per mL per vial in cell-freezing media (90% Fetal Bovine Serum (FBS), 10% Dimethyl Sulfoxide (DMSO)). Samples were thawed in batches for analysis.

Cytokines were quantitated in baseline and on-treatment plasma samples following manufacturer instructions for V-PLEX Human Proinflammatory Panel 10-plex kits (Meso Scale Diagnostics) and a 3-plex custom panel including IFN-α, IFN-β and IP-10. Resulting light intensity was read using an MSD QuickPlex SQ 120 imager. Each analyte's concentration was calculated from its 4-parameter logistic fit calibration curve generated using the standards.

Flow cytometry was performed on cryopreserved PBMCs using a T-cell activation/exhaustion marker panel. Briefly, human PBMC samples were thawed, washed, counted, and stained with a Fixable Aqua Viability Dye (Invitrogen) and a cocktail of antibodies to the following surface markers: CD8-Qdot605 (Invitrogen, 3B5), CD4-Qdot 655 (Invitrogen, S3.5), PD-1-PE (BD, MIH4), LAG-3-FITC (Enzo, 17B4), ICOS-PE-Cy7 (eBioscience, ISA-3), TIM-3-APC (R&D Systems, 344823). Cells were next fixed and permeabilized with the FoxP3/Ki-67 Fixation/Permeabilization Concentrate and Diluent (eBioscience), then stained intracellularly with CD3-BV570 (BioLegend, UCHT1), Ki-67-AlexaFluor700 (BD, B56), FoxP3-eFluor450 (eBioscience, PCH101), and CTLA-4-PerCP-eFluor710 (eBioscience, 14D3). Stained cells were analyzed on a BD Biosciences LSRFortessa using FlowJo software (FlowJo). Isotype control stains were used to establish positivity gates for PD-1, LAG-3, ICOS, TIM-3, FoxP3, and CTLA-4.

ctDNA analysis

Peripheral blood samples were drawn from patients into two streck cell-free DNA (cfDNA) Blood Collection Tubes (Streck, La Vista, Nebraska, USA). cfDNA was extracted using the QIAsymphony SP system (Qiagen). The extracted cfDNA was quantified and stored in accordance with validated protocols.[24]

cfDNA samples were analyzed using the MSK-ACCESS assay, which identifies mutations and select copy number alterations across 129 genes[17 25] at a coverage depth exceeding 15,000× to detect allele frequencies as low as 0.1%.[17] MSK-ACCESS detects copy number alterations (CNAs) in a limited set of genes, including *AR, BRCA1, BRCA2, CDK4, EGFR, ERBB2, MET, MDM2, MLH1, MSH2, MSH6, and MYC.*

From BAM files, variants were called using GetBaseCountsMultiSample (V.1.2.5). Calling of somatic single-nucleotide variants and short insertions/deletions (indels) required ≥2 duplex consensus reads from both DNA strands, as outlined in prior work.[17] De novo variants were reviewed using the Integrative Genomics Viewer to exclude sequencing artifacts, and a fragment analysis tool was used to filter out variants associated with clonal hematopoiesis. Using variants identified in the archival tissue as a reference, ctDNA at each time point was evaluated to determine positive or negative status. The average variant allele frequency was calculated for all somatic mutations categorized as clonal or subclonal in the archival tissue sample, ensuring that they were represented in the MSK-ACCESS panel.

RNA sequencing

RNA was extracted from archival FFPE samples and flash-frozen biopsy samples. RNA sequencing was performed by PicoImmune (https://picoimmune.com) using the NanoString nCounter Human PanCancer IO 360 Panel. Raw NanoString nCounter data (.NCC files) were processed and normalized using the *processNanostringData* function from the NanoTube library in R.[26 27] Immune cell populations were quantified using the immunedeconv R package and its deconvolute function with MCP-counter.[28] For each patient, a tertiary lymphoid structure (TLS) score was calculated using the Single-sample Gene Set Enrichment Analysis (ssGSEA) method and the expression of 11 literature-derived genes: CCL2, CXCL11, CXCL10, CXCL9, CCL18, CCL21, CCL4, CXCL13, CCL5, CCL19, and CCL8.[29 30] Scores were categorized as TLS-high or TLS-low, using the median as a cut-off.

Statistical analysis

Data cut-off was July 28, 2023. Using a one-stage design to test the alternative hypothesis that the ORR was ≥0.25 versus the null hypothesis that ORR was ≤0.05, there was 91% power and 8% probability of type 1 error. If ≥3 patients had a response by 24 weeks, the treatment would be considered effective.

PFS was defined as time from treatment initiation to progression per RECIST or by clinical assessment,

death due to any cause, treatment-related toxicity, or last follow-up, whichever occurred first. OS was defined as the time from treatment initiation to death due to any cause or last follow-up. Baseline cytokine and flow cytometry measurements were summarized using median and IQR. Kaplan-Meier methods were used to estimate PFS and OS, both overall and according to levels of immune markers dichotomized at the median value. Fisher's exact test and Wilcoxon rank-sum test were used to test the association of immune markers (at baseline and change from baseline to week 9) with a binary 90-day progression endpoint. Cox proportional hazard models were used to test the association between clinical factors and PFS and OS. Analysis of treatment-associated change in immune markers was restricted to patients who had not yet progressed by week 9.

FGA and TMB were compared between patients with PFS of ≥16 weeks versus <16 weeks using Fisher's exact test. Gene expression was compared between the same groups using the *runLimmaAnalysis* linear model function within NanoTube. Expressed genes were ranked based on their log fold difference in expression between groups. The ClusterProfiler package in R was used to assess whether genes within the Immunogenic gene signature set (C7 MSigDB; https://www.gsea-msigdb.org/gsea/msigdb/) displayed coordinated changes in expression.[31] Genes with a p value<0.05 after false discovery rate (FDR) correction were considered statistically significant. Heatmaps were generated from scaled data using the ComplexHeatmap function in R to visualize the expression patterns of identified genes in each analysis. Differences in immune cell proportions between patients based on PFS groups were determined by fitting a linear model with PFS group as the predictor variable and evaluated by analysis of variance. SAS V.9.4 (V.9.4 SAS Institute, Cary, North Carolina, USA) and R (V.4.3.1; R Foundation for Statistical Computing, Vienna, Austria) were used for all analyses. All tests were two-sided, and p<0.05 was considered significantly different.

RESULTS

Patients and treatment

Between November 5, 2020, and June 9, 2022, 20 patients were enrolled and treated. Median age was 58 years (range, 42–78) (table 1). Most patients were female (85%) and had a uterine primary. Median number of prior lines of therapy was 2. At data cut-off no patients remained on study.

Table 1 Patient demographics and clinical characteristics.

	n=20
Age (years)	58 (42–78)
Gender	
Female	17 (85%)
Male	3 (15%)
Body mass index (median, range)	25.4 (19.5–31.4)
Site	
Uterine	16 (80%)
Soft tissue	4 (20%)
ECOG performance status	
0	11 (55%)
1	9 (45%)
Prior lines of therapy	2 (1–3)

Median (range) for continuous variables.
ECOG, Eastern Cooperative Oncology Group.

Efficacy

The best ORR by 24 weeks in this study was 5% (95% CI 0% to 25%), with one PR (59% decrease) in a patient with uterine LMS and a somatic *BRCA2* deep deletion (figure 1A). This patient had a sustained response through 11 months of therapy, when she developed grade 3 renal dysfunction and treatment was held. Steroids were initiated, and despite restoration of kidney function to baseline levels, protocol therapy was permanently discontinued.

Eight patients (40%) had RECIST progression of disease at their first assessment at 8 weeks (figure 1B). Of these 8, three patients had one or both drugs held due to toxicity (elevated liver enzymes, fever, pneumonitis) at the time of imaging. One additional patient without RECIST assessments was considered a non-responder due to rapid clinical progression.

Ten patients had stable disease (SD) by RECIST at the time of first restaging scan (figure 1B). However, in four of these patients, protocol therapy was discontinued for clinical progression, and one patient with baseline mild renal insufficiency stopped treatment due to worsening kidney function. Reasons for discontinuation from study therapy in the five patients who continued treatment included: RECIST progression (n=2), clinical progression, toxicity in the form of autoimmune hemolytic anemia, and withdrawal of consent.

Median PFS was 7.8 weeks (95% CI 6.7 to 15.0) for the overall population and did not differ between the soft tissue LMS and uterine LMS subgroups (soft tissue LMS 7.8 (95% CI 6.7 to NR (not reached)) vs uterine LMS 7.5 (95% CI 6.4 to 15.0); p>0.95) (figure 1C). PFS at 24 weeks was 25% (95% CI 9% to 45%). Median OS was 43 weeks (95% CI 22.3 to 97.1) for the overall population (figure 1D). While median OS was longer among soft tissue LMS versus uterine LMS patients, 95% CIs mostly overlapped (97.1 weeks (95% CI 38.7 to 98.1) vs 43 weeks (95% CI 22.3 to 90); p=0.810). OS at 24 weeks was 75% (95% CI 50% to 89%). There were no statistically significant differences in PFS or OS based on sex, body mass index, or the number of prior lines of therapy.

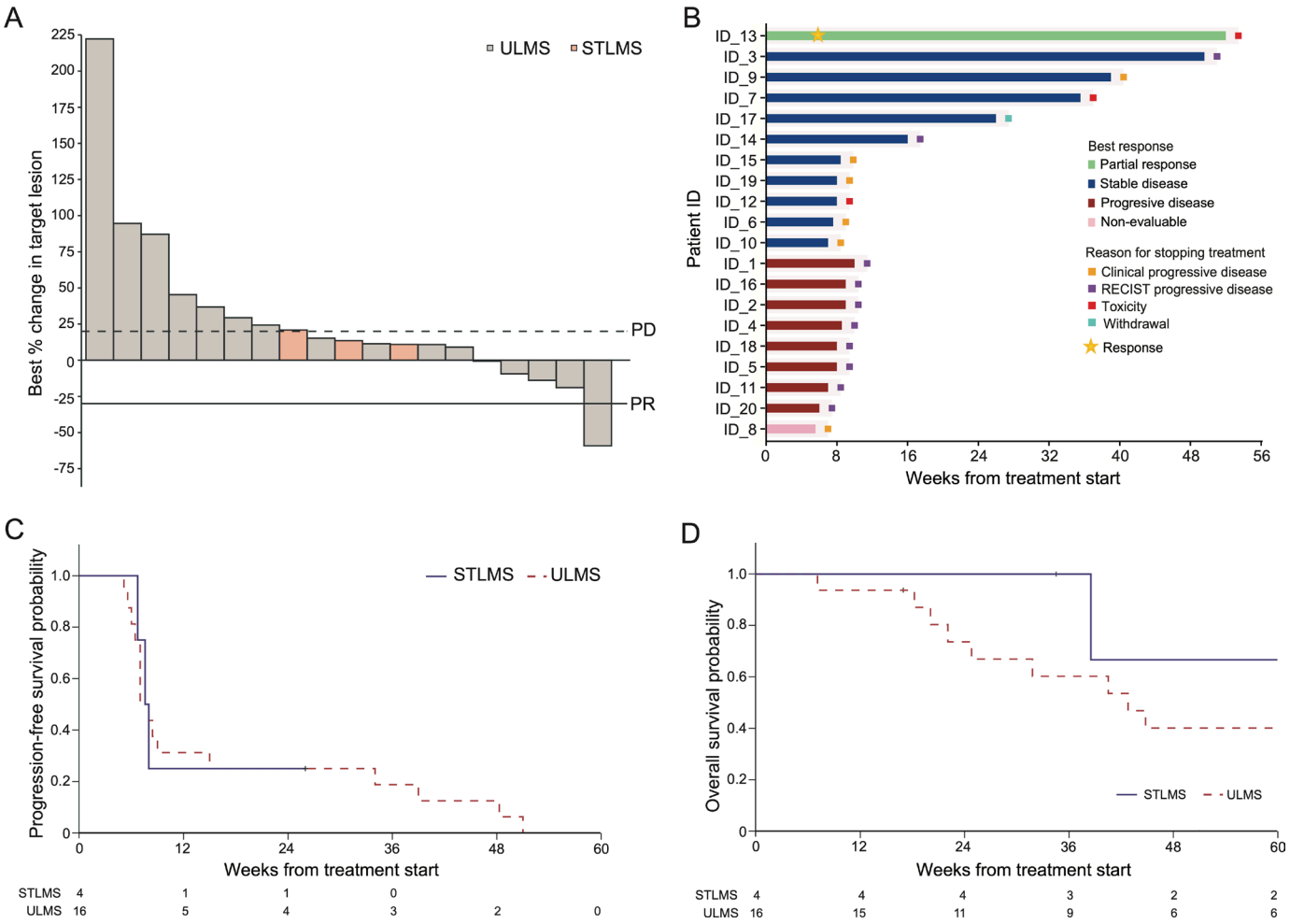

Figure 1 Treatment outcomes. (A) Best tumor response by RECIST; (B) duration of treatment; (C) progression-free survival by primary site; (D) overall survival by primary site. RECIST, Response Evaluation Criteria in Solid Tumors; PD, progressive disease; PR, partial response; STLMS, soft tissue leiomyosarcoma; ULMS, uterine LMS.

Safety

19 (95%) patients had a treatment-related adverse event (TRAE) and 7 (35%) had a grade 3 or higher TRAE (table 2). The most common TRAEs overall were fatigue (9; 45%), nausea (8; 40%) and diarrhea (7; 35%). The most common grade 3 or 4 TRAEs were febrile neutropenia (2; 10%), leukopenia (2; 10%), and pneumonitis (2; 10%).

As noted above, three patients permanently discontinued all study therapy for toxicity: renal dysfunction in two and autoimmune hemolytic anemia in one. Two patients had grade 3 pneumonitis attributed to either nivolumab or rucaparib. One patient fully recovered following drug interruption and administration of steroids; they were not further treated as subsequent imaging showed disease progression. The other patient was treated empirically with steroids for presumed pneumonitis but died due to concomitant rapid progression of lung disease. Patients whose toxicity was attributed to one study drug were permitted to continue the other as single-agent therapy. Reasons for individual study drug discontinuation included grade 2 or 3 elevated liver enzymes attributed to nivolumab (n=2) and persistent grade 2 fever attributed to rucaparib (n=1). All patients requiring drug discontinuation recovered fully from the toxicity with supportive care and steroids as required per protocol. There were no deaths related to TRAEs.

In 14 patients (70%) dose delays were required due to toxicity and 8 patients (40%) required dose reduction of rucaparib due to elevated liver enzymes, leukopenia or neutropenia, fatigue, or fever.

Targeted tumor and germline sequencing

MSK-IMPACT was performed on 19 of 20 archival specimens (figure 2). Two (10%) patients had *BRCA2* deep deletions on molecular profiling (one patient with co-occurring *PTEN* homozygous deletion and *RAD51* intragenic deletion; best response of SD). The patient with SD (−19%) developed severe myelosuppression, and during drug hold, the disease progressed. Other notable alterations in HR and/or DDR genes were *BRIP1* intragenic deletion (co-occurring with *RB1* and *TP53* homozygous deletions; this patient had SD) and *RAD51* intragenic

Table 2 Treatment-related adverse events by grade. Events listed include those occurring at a frequency of ≥20% for any grade or ≥10% at grade 3 or 4

Adverse event	Grade 1 or 2	Grade 3 or 4	Total
Blood and lymphatic			
Febrile neutropenia	0	2 (10%)	2 (10%)
Leukopenia	2 (10%)	2 (10%)	4 (20%)
Gastrointestinal			
Abdominal pain	4 (20%)	0	4 (20%)
Vomiting	5 (25%)		5 (25%)
Nausea	8 (40%)	0	8 (40%)
Diarrhea	7 (35%)	0	7 (35%)
General			
Fatigue	8 (40%)	1 (5%)	9 (45%)
Hepatobiliary			
AP increased	3 (15%)	1 (5%)	4 (20%)
ALT increased	3 (15%)	1 (5%)	4 (20%)
AST increased	4 (20%)	1 (5%)	5 (25%)
Renal			
Creatinine increased	4 (20%)	0	4 (20%)
Metabolism			
Anorexia	6 (30%)	0	6 (30%)
Nervous system			
Dysgeusia	6 (20%)		6 (30%)
Respiratory			
Pneumonitis	0	2 (10%)	2 (10%)

Events listed include those occurring at a frequency of ≥20% for any grade or ≥10% at grade 3 or 4.
AP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

deletion (in one additional patient with *RB1* homozygous and *BRCA2* deletions; best response of SD).[19]

Mean TMB was low at 0.8 (range 0–7). All tumors were mismatch repair-proficient, except one for which indeterminate status was noted. Follow-up confirmatory immunohistochemistry was not completed.

15 patients had germline testing. Germline variants annotated as pathogenic or likely pathogenic were identified in five patients, including likely pathogenic *NBN* and *MYC* variants in the patient with PR. A pathogenic *MSH2* variant was detected in one patient treated for 11 months. *REL*, *RECQL*, and *FANCA* germline variants were also detected in three patients who did not derive meaningful benefit from treatment (figure 2).

Although not statistically significant, PFS was greater in patients with tumor alterations in the *BRCA2* gene at 42.5 weeks versus 7.3 weeks (p=0.088). However, there was no difference in PFS or OS based on overall HR deficiency/DDR status. While median TMB was higher in patients experiencing a PFS>16 weeks, and FGA was higher in patients with PFS ≤16 weeks, the differences were not significant. No significant differences in mutations or copy number alterations were found to be associated with PFS or best ORR.

ctDNA analysis

A total of 19 patients (all time points) had matched tumor sequencing using the MSK-IMPACT assay that demonstrated alterations in regions included in the MSK-ACCESS panel. Among these, 13 (68%) patients had ≥1 variant detected by both assays. Five (26%) patients had all variants detected exclusively by MSK-ACCESS, and 1 (5%) patient had all variants detected exclusively by MSK-IMPACT (figure 3A,B). ctDNA was detected in plasma in 18 patients at baseline (online supplemental figure 1); the most frequently mutated genes were *TP53, NF1, and MED12*. The total number of CNAs detected in the restricted gene set with MSK-IMPACT was 7 (37% of patients) compared with 2 (11% of patients) using MSK-ACCESS.

10 patients discontinued study treatment due to RECIST progression of disease. ctDNA was detectable in nine of these patients at baseline; in eight patients the ctDNA level was increasing at the end of treatment (figure 3B). Among the five patients with RECIST SD in whom treatment was discontinued due to clinical decline, ctDNA was increasing at treatment discontinuation in four (figure 3C). One patient who was on treatment for 9 months presented with diplopia and headache shortly after the imaging visit (RECIST: −12%; ctDNA increasing) and was found to have a new skull metastasis. Among the four patients who discontinued study therapy for reasons of toxicity or withdrawal of consent, ctDNA was detectable at baseline for three (figure 3D). In the patient with PR, ctDNA became undetectable at week 5 until the end of treatment when therapy was discontinued due to toxicity. The second patient with somatic *BRCA2* deletion and minor response had ctDNA that was undetectable or decreasing from baseline until treatment was held due to anemia. ctDNA trend was increasing shortly thereafter, though RECIST-classified response remained SD.

Cytokines and flow cytometry

18 patients had baseline plasma samples available for cytokine analysis. None of the 13 analytes tested at baseline were associated with differences in median PFS or 90-day progression. 17 patients had paired samples at baseline and on-treatment. IFN-α, IFN-β, and interleukin (IL)-4 results were below the limit of quantitation across all samples. IFN-γ, IL-10, IL-8, Tumor Necorsis Factor (TNF-α), and Interferon Gamma-induced Protein 10 (IP-10) increased with treatment, and increases in IP-10, IL-10, and IFN-γ appeared to correlate with progression at 90 days, but none of these results were statistically significant (online supplemental figure 2A–D). A clear pattern in cytokine expression levels over time was not evident for the two patients with BRCA2-deleted tumors. In the patient with SD, levels of IFN-γ, IL-10, TNF-α, and

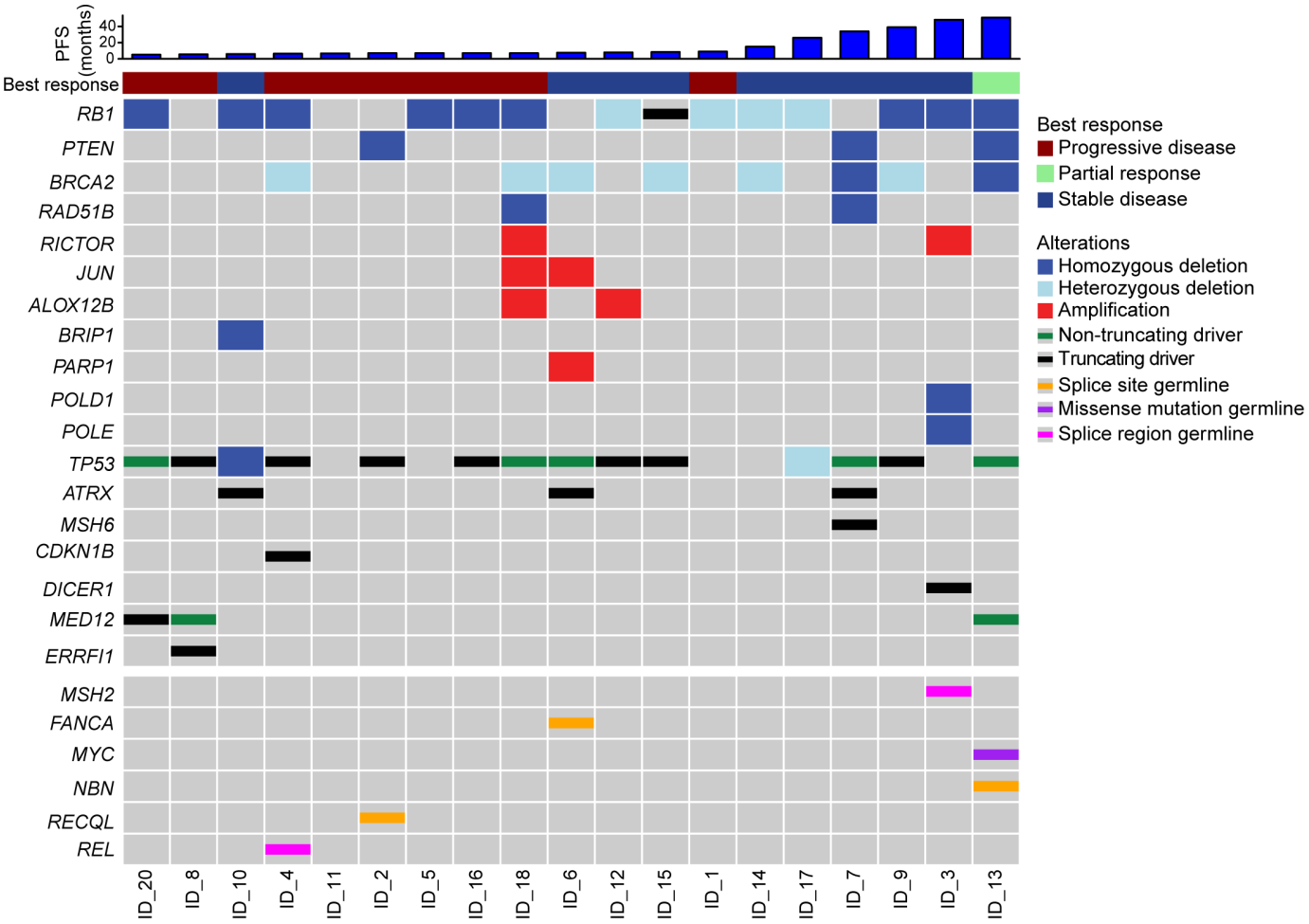

Figure 2 Oncoprint of targeted tumor and germline sequencing. Oncoprint summarizing targeted somatic next-generation sequencing (MSK-IMPACT) and germline sequencing results in the study participants. Top, progression-free survival (PFS) in months and best overall response. Bottom, germline mutations.

IP-10 increased at the end of treatment compared with earlier time points (data not shown). In the PR, these cytokines also peaked at the week 9 time point (RECIST: −33.8%, ctDNA undetectable). However, the cytokine levels at these time points were not significantly different from those observed in other patients across various time points, making the significance of these changes unclear.

All 20 patients had PBMCs available for flow cytometry T-cell analysis and 19 patients had paired baseline and on-treatment samples. There was no association between the abundance of any T-cell type at baseline and 90-day PFS. Patients who had higher baseline circulating FOXP3+CD8+ T cells had better PFS (9 weeks (95% CI 6.7 to 48.3)) compared with those with numbers below the median (7 (95% CI 5.1 to 8.4) weeks, p=0.027), as did patients with higher baseline circulating CD3+T cells at baseline or lower baseline CD3+/CD4+ratio, although not significant, (CD3+T cells below median 7 weeks (5.1–8.4) versus above median 11.5 weeks (6.63–48.3), p=0.051; CD3+/CD4+ratio 11.7 (5.6–39) vs 7 (5.1–8), 0.083) (online supplemental figure 2E,F). TIM3+CD8+ T-cell increase from baseline to 9 weeks was significantly greater among those with progression by 90 days, (median (IQR) 0.9 (0.3 to 1.7) vs −0.1 (−0.2 to 0.5), p=0.025) (online supplemental figure 2G).

Gene expression profiling

RNA expression profiling was available from 13 archival specimens and 6 on-treatment biopsies, including 4 matched pairs. 15 genes were differentially expressed at baseline in patients who derived benefit from treatment (at least SD by 16 weeks; n=5) versus those who did not (progression of disease prior to 16 weeks; n=8) (figure 4A). Among these, three genes were upregulated: *CCL5* (encoding a chemokine), *SRFP1* (encoding a Wnt signaling protein), and *SNCA*. In four of five patients in the benefiting group, *BRCA2* was downregulated; however, no gene expression differences were statistically significant after FDR p value adjustment.

Patients who benefited from treatment displayed a statistically significant upregulation of IFN-α and IFN-γ hallmark pathways in both baseline (adjusted p=0.005 for IFN-α, adjusted p=0.03 for IFN-γ) (figure 4B, online supplemental table 1) and week 8 on-treatment biopsies (adjusted p=0.0002 for IFN-α, adjusted p=0.0001 for IFN-γ; online supplemental figure 3A, table 1).

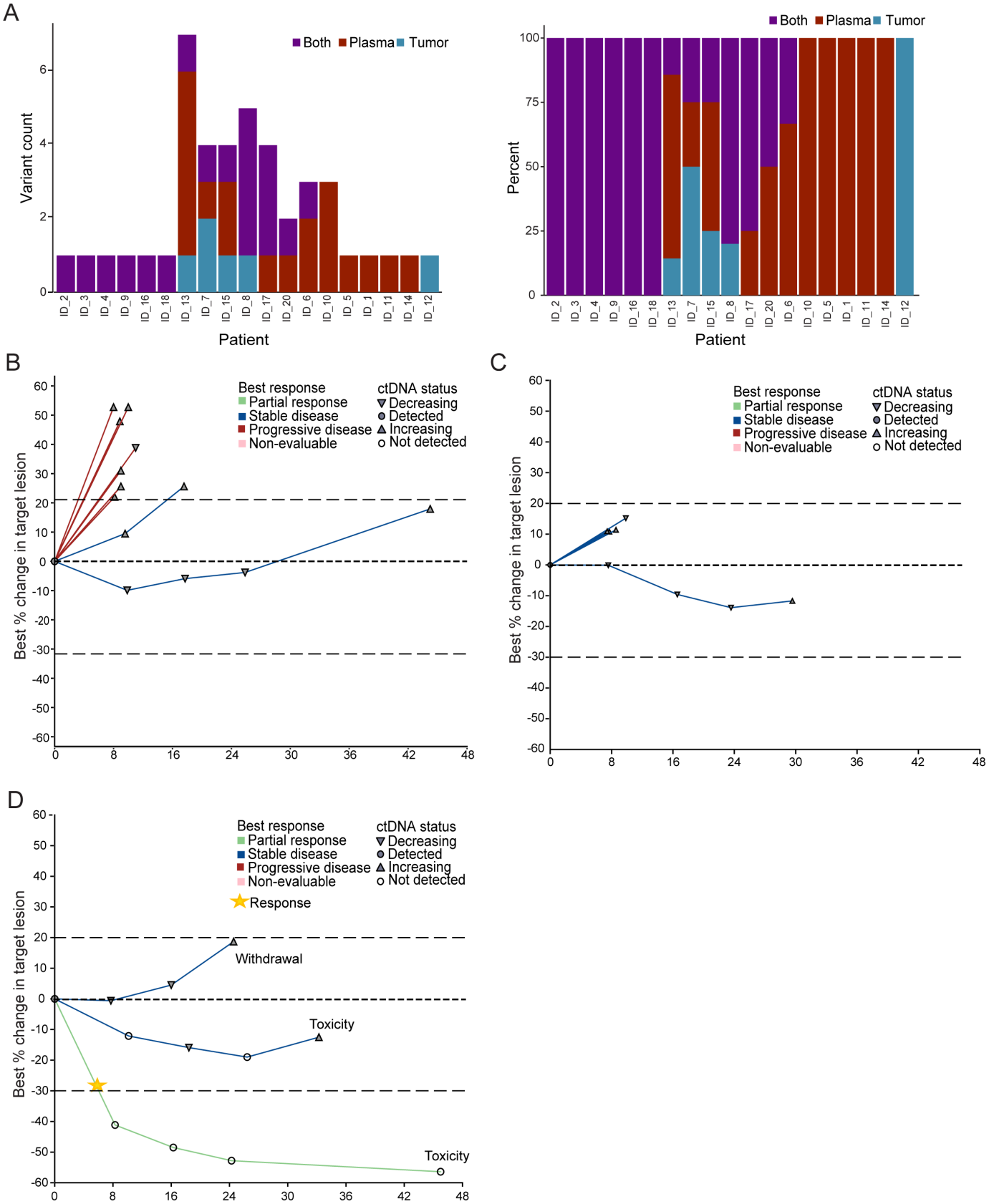

Figure 3 Circulating tumor DNA (ctDNA) results. (A) Concordance between ctDNA and targeted tumor sequencing by count (left) and percent (right) in 19 patients with MSK-IMPACT performed on archival tumor specimens; ctDNA variant calls at any time point are shown. (B–D) Spider plots of responses of patients who discontinued study due to (B) RECIST progression, (C) clinical progression, or (D) toxicity or withdrawal of consent, each annotated with ctDNA changes. RECIST, Response Evaluation Criteria in Solid Tumors.

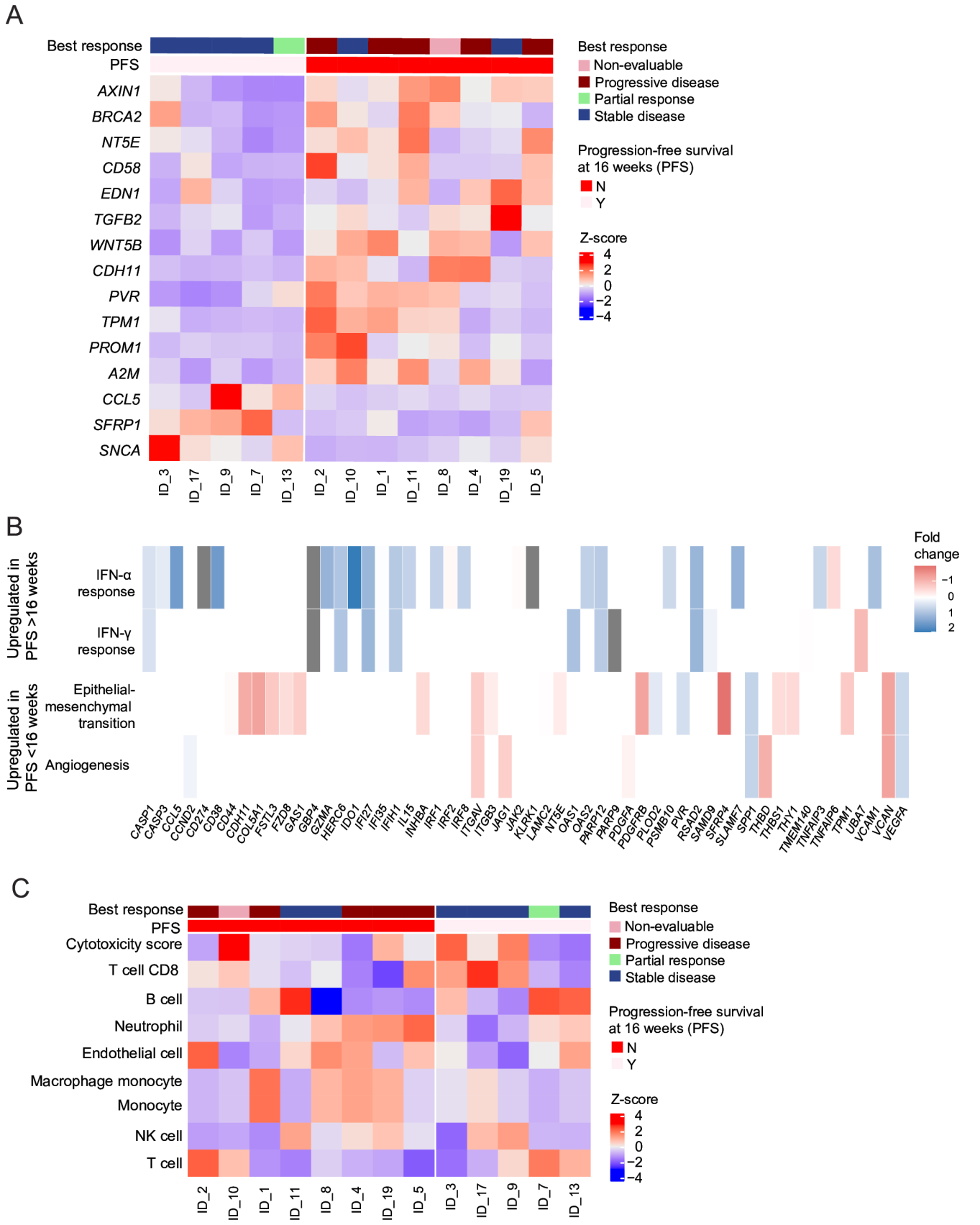

Figure 4 Gene expression. (A) Heatmap of relative expression at baseline of genes that differed significantly by nominal p value between patients with versus without progression at 16 weeks. (B) Heat plot of expression of enriched hallmark pathways that were upregulated or downregulated at baseline in patients with PFS≥16 weeks compared with levels in patients with PFS<16 weeks. (C) Heatmap of enrichment of immune and stromal cell populations at baseline between patients with versus without progression at 16 weeks. IFN, interferon; NK, natural killer.

Epithelial-mesenchymal transition (EMT) and angiogenesis hallmarks were downregulated at baseline among patients with PFS<16 weeks (adjusted p=0.005 for both pathways) (figure 4B, online supplemental table 1). EMT was also downregulated in the on-treatment samples from patients with PFS<16 weeks (adjusted p=0.0008, online supplemental figure 3A, table 1).

While transcriptomic analysis using MCP-counter revealed small differences in tumor immune and non-immune stromal cell populations at baseline (figure 4C) and on-treatment (online supplemental figure 3B) between patients with PFS ≥16 weeks and those with PFS<16 weeks, none of these differences reached statistical significance. There was no significant difference in TLS signature expression at baseline between PFS≥16 weeks versus PFS<16 weeks (p=0.2). PFS was 21 weeks in TLS-high versus 7 weeks in TLS-low (p=0.9)

DISCUSSION

This study did not demonstrate meaningful activity in LMS for the combination of rucaparib and nivolumab. The only clinical benefit was noted in patients with uterine LMS and *BRCA2* deep deletions (one PR and one SD consisting of target lesion decrease of 19% and initial clinical response). ctDNA was also undetectable in these two patients as they responded. Four additional patients remained on treatment for ≥16 weeks, including one patient with non-uterine LMS. The results of this study are comparable to those of the DAPPER study, in which a 3% ORR was noted in patients with LMS treated with olaparib and duvalumab.[32]

There was no objective response to therapy in patients with alterations in other DDR or HR deficiency genes. This is similar to the recently published pan-cancer study of the PARP inhibitor talazoparib in combination with the PD-L1 inhibitor avelumab, where activity was noted in *BRCA1/2*-altered, but not in patients with other DDR alterations such as *ATM*.[33 34] On the other hand, in a phase 2 study of olaparib and temozolomide in uterine LMS, patients with somatic alterations in *RAD51B, PALB2, and ATR* appeared to have improved PFS; results from the confirmatory study are awaited.[35]

Pathogenic alterations in *BRCA1/2* genes have been reported in only 4–7% of LMS overall and up to 10% of uterine LMS,[36 37] with a higher detection rate by whole exome sequencing.[8] Though this study enrolled patients with LMS from any primary site, 70% of the study population had uterine primary LMS. Emerging data suggests that in uterine LMS, somatic biallelic *BRCA2* loss may confer BRCA dependence, similar to ovarian and breast cancers.[33 38] No patients with a primary soft tissue LMS tumor that harbored *BRCA* alterations enrolled. Therefore, the phenotypic and therapeutic relevance of *BRCA* alterations in non-uterine LMS is unclear. HR deficiency scores measuring genomic scarring have been investigated but do not appear to correlate with PFS or OS in LMS[37] and are not predictive of response to PARP inhibitor therapy in ovarian cancer.[39] Notably, though only two patients in our study had somatic *BRCA2* deletions, low RNA expression of *BRCA2* was noted in four of five patients who clinically benefited. This finding could be further explored as a potential biomarker of benefit to PARP inhibition.

Our hypothesis was that PARP inhibition would favorably modulate the tumor immune microenvironment and lead to improved outcomes with PD-1 inhibitors in LMS. We did not observe a positive association between changes in immune cell infiltration or activity and clinical outcome. Prior work suggests that HR deficiency correlates with the presence of macrophages, particularly M2-polarized macrophages, in the tumor microenvironment, which could reflect immunosuppression and explain the lack of robust activity seen in this trial.[5] Type 1 and type 2 IFN pathways did appear to be upregulated in baseline and on-treatment tumor samples from patients with SD beyond 16 weeks. Notably, in ovarian cancer, baseline type 1 IFN signaling was enriched in patients with responses to the combination of niraparib and pembrolizumab.[39] In our study, upregulation of blood cytokines after treatment was negatively associated with prognosis, but this was not statistically significant, and some levels were below the level of detection at baseline. Unfortunately, we were only able to collect a few on-treatment biopsies, as many patients withdrew consent on disease progression. Therefore, whether PARP inhibition specifically upregulated the IFN pathway in the tumors of patients who clinically benefited remains unclear. Elevated expression of the epithelial marker, E-cadherin, has been noted in a subset of LMS and is associated with improved survival.[40] We noted the opposite pattern in our study, with downregulation of EMT associated with worse outcomes, underscoring the need for a larger analysis.

This study highlighted a common clinical challenge of using RECIST to assess response in LMS. Five (25%) patients in this study discontinued protocol therapy due to clinical progression despite a RECIST assessment of SD. Four of these patients had increasing ctDNA levels at that time point. Detectable ctDNA levels at baseline and after two cycles of chemotherapy have been associated with worse survival in LMS.[41] In our study, almost 90% of patients with disease progression had increasing ctDNA levels, suggesting that ctDNA may be a useful adjunctive tool. However, the utility of ctDNA assessment is limited by its lower sensitivity for identifying deletion events because of background noise from normal cfDNA.

Toxicity in this study was high with 95% of patients experiencing a TRAE and 35% of patients experiencing a grade 3 or higher TRAE. Though this toxicity rate is similar to other studies of this combination[33] and other immunotherapy combinations,[42] dose delays or treatment discontinuation may have contributed to this trial's outcome; 7 (35%) patients were either having drug held at the time of first assessment or discontinued therapy due to toxicity. Rucaparib and nivolumab can cause overlapping toxicities such as elevated kidney and hepatic

function and pneumonitis, rendering toxicity attribution and management challenging. Despite detailed protocol-specific guidelines, clinical uncertainty remained and treating physicians opted to discontinue protocol therapy for patient safety in several instances.

In conclusion, there was no meaningful response to rucaparib and nivolumab in patients with LMS in this study, suggesting that the addition of a PARP inhibitor does not improve on low benefit rates to PD-1 inhibitors in LMS. Only one RECIST response was noted, which occurred in one of the two patients with somatic *BRCA2* deletions. Unfortunately, time on study for these two patients was limited due to the development of toxicity. Four additional patients had disease benefit for at least 16 weeks. Whether differences in efficacy among patients reflect *BRCA* alteration dependence, varying upregulation of IFN pathways by PARP inhibition, or other disease variables remains unclear.

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Contributors SM, ER, and CMK designed the study. KS performed the statistical analysis. MB, KC, RS, and PW performed correlative analyses. SM, ER, CMK, SMC, MLH, VA, LBB, JEC, PC, SD'A, MAD, MMG, MLK, RGM, AG, VM, and MMR recruited and/or treated patients. L-XQ developed the statistical plan and supervised the analyses. SS and J-MC provided database and correlative project support. RAL performed the radiological assessments for the study. JE supervised and/or performed on-study biopsies. SM, KS, MB, KC, and PW wrote the manuscript. SM, KS, MB, KC, PW, L-XQ, and RS discussed and interpreted the results. All authors reviewed the revised manuscript. SM is the guarantor.

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Competing interests SM has provided consulting/advisory services for Deciphera and received research funding for clinical trials via the institution from Ascentage Pharma, Clovis, Bristol Myers Squibb, Jazz, Pfizer/Trillium, PTC Therapeutics, and Hutchinson Medipharma. ER has received funding for clinical trials via the institution from Iovance Biotherapeutics, Incyte Corporation, and Arcus Biosciences and reports stock ownership from Iovance Biotherapeutics and PMV Pharma. CMK has provided consulting/advisory services for Deciphera, received funding for clinical trials via the institution from Merck, Amgen, Inhbrx, IXRx, Curadev, Regeneron, and Servier and has a spouse employed at Daichii Sankyo. MLH has provided consulting/advisory services for AADi and Targeted Oncology and has a spouse employed by Sanofi until Feb. 2024. JEC has received research funding from ONO. PC has provided consulting/advisory services for Deciphera, Ningbo NewBay Medical and has received funding for clinical trials via the institution from Pfizer/Array, Deciphera, and Ningbo NewBay Medical Technology. SD'A has provided consulting/advisory services for Ratio, Adaptimmune, GI Innovation, EMD Serono, Amgen, Nektar, Immune Design, GSK, Incyte, Immunocore, Aadi, Pfizer, Servier, and Rain Theraputics and has received funding for clinical trials via the institution from Incyte, Merck, EMD Serano, Nektar, and Deciphera. MAD has received funding for clinical trials via the institution from Eli Lilly, AADi, and Sumitomo. MMG has provided consulting/advisory services for Epizyme, Ayala Pharmaceuticals, Rain Therapeutics, AADi, Ikena Oncology, and Kura Oncology, honoraria from Medscape, Guidepoint Global, Med Learning Group, Research to Practice, Great Debates and Updates, GLG, OncLive/MJH Life Sciences, and MJH/PER, received funding for clinical trials via the institution from Ayala Pharmaceuticals, AADi, Athenex, Boehringer Ingelheim, Foghorn Therapeutics, Ikena Oncology, GlaxoSmithKline, Rain Oncology, Regeneron, SpringWorks Therapeutics, SERVIER, Tango Therapeutics, Kymera, Erasca, and Vivace Therapeutics, has patents/ royalties/ other intellectual property from UpToDate and GODDESS PRO Desmoid Tumor, travel/accommodations/expenses from Epizyme, and uncompensated relationship with Foundation Medicine. RGM has provided consulting /advisory services for AADi, Boehringer Ingelheim, Deciphera, and Peel Therapeutics, received royalties from UpToDate, received honoraria from the American Society of Clinical Oncology, and has equity ownership/stock options in Peel Therapeutics. AG has provided consulting/advisory services for Merck, Menarini/Stemline, received funding for clinical trials via the institution from Aadi, Lilly, Merio BioPharma and grant support from ASCO. VM has received research funding via the institution from AstraZeneca, Bristol Myers Squibb, Clasi, Cullinan Oncology, DualityBio, Eisai, Faeth Therapeutics, Karyopharm Therapeutics, Merck, Takeda, and Zymeworks; received travel support from Eisai and Merck; and provided consulting/advisory services for Clovis Oncology, Cullinan Oncology, DualityBio, Eisai, Faeth Therapeutics, GlaxoSmithKline, Immunocore, iTeos Therapeutics, Karyopharm Therapeutics, Lilly, Merck, Mereo BioPharma, MorphoSys, MSD, Novartis, Regeneron, Sutro Biopharma, and Zymeworks. JE has provided consulting services for AstraZeneca. WT has provided consulting/advisory services for AADi, Abbisko, Amgen, AmMax Bio, Avacta, Bayer Pharmaceuticals, BioAtla, Boehringer Ingelheim, C4 Therapeutics, Certis Oncology, Cogent, Curadev, Daiichi Sankyo, Deciphera, Ikena, IMGT, Inhibrx, Innova, Ipsen, PharmaEssentia, Ratio, Recordati, Servier, Sonata, and Synox; owns stock in Certis Oncology; and co-founded and owns stock in Atropos Therapeutics. All other authors report no relevant conflicts of interest.

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