Nivolumab and Ipilimumab for Metastatic Castration-Resistant Prostate Cancer With an Immunogenic Signature: The Multicenter, Two-Cohort, Phase II NEPTUNES Study

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ABSTRACT

PURPOSE Efficacy of immune checkpoint inhibitors in unselected patients with metastatic castration-resistant prostate cancer (mCRPC) is limited. The NEPTUNES study evaluated combination nivolumab and ipilimumab in patients with immunogenic signature–positive (ImS+) mCRPC.

MATERIALS AND METHODS This open-label, 2-cohort, phase II trial enrolled patients with ImS+ mCRPC progressing on ≥1 previous line of treatment. ImS+ was defined by (1) mismatch repair deficiency (MMRD); (2) DNA damage repair gene loss; and/or (3) high inflammatory infiltrate (HII). Patients received four doses of nivolumab 1 mg/kg + ipilimumab 3 mg/kg (C1) or nivolumab 3 mg/kg + ipilimumab 1 mg/kg (C2) followed by nivolumab 480 mg once every 4 weeks up to 10 cycles. The primary end point was composite response rate (CRR) assessed radiologically, biochemically, and by reduction of circulating tumor cells. Secondary end points included toxicity, progression-free survival, overall survival, and duration of response.

RESULTS Between May 2018 and June 2022, 35 (C1) and 36 (C2) patients commenced treatment. The CRR in C1 was 14/35 (40%, 90% CI, 26% to 55%) and in C2 was 9/36 (25%, 90% CI, 14% to 40%). The overall CRR was 23/71 (32%, 90% CI, 23% to 43%). Response rates were higher in patients with MMRD (7/10), BRCA2 loss (4/8), and HII ± other ImS+ features (13/30). Duration of response for patients with HII without other ImS+ features, DNA repair gene loss without MMRD, and MMRD was 2.6, 17.3, and 10 months, respectively. Grade 3 to 4 treatment-related adverse events occurred in 22/35 (63%) in C1 and 12/36 (33%) patients in C2. There were no treatment-related deaths.

CONCLUSION Nivolumab 1 mg/kg + ipilimumab 3 mg/kg is an active treatment in ImS+ pretreated mCRPC. Nivolumab 3 mg/kg + ipilimumab 1 mg/kg has less toxicity but may have lower efficacy. HII is a promising prospectively tested predictive biomarker in prostate cancer that could be integrated into future trials.

ACCOMPANYING CONTENT

Data Supplement

Protocol

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INTRODUCTION

Prostate cancer is the most common cancer in men and, despite new treatments, mortality rates remain high.[1] Checkpoint inhibitor (CPI) therapy has changed the management of many tumors but response rates in metastatic prostate cancer are low. In unselected patients with metastatic castration-resistant prostate cancer (mCRPC), CPI monotherapy has led to radiologic or biochemical responses in <10% of patients,[2,3] whereas combination of nivolumab and ipilimumab has led to a 10%-25% response rate.[4,5] Given the well-recognized toxicities of combination CPI therapy, this level of activity has been deemed insufficient for clinical benefit. Despite these underwhelming results, exceptional responders to CPI have been reported.[3]

Prostate cancer has an immunosuppressive tumor microenvironment characterized by low inflammatory infiltrate,

CONTEXT

Key Objective

To determine whether selection of patients with metastatic castration-resistant prostate cancer according to an immunogenic signature, which included tumor immune infiltrate, enriches for responses to a combination of immune checkpoint inhibitors compared with previous trials in unselected populations.

Knowledge Generated

NEPTUNES reports higher response rates for selected patients treated with nivolumab and ipilimumab compared with previous trials, with some patients achieving long durable responses. To our knowledge, this is the first trial that selects patients with prostate cancer on the basis of the presence of high immune infiltrate, which, as a standalone marker, results in a promising response rate of 43%.

Relevance *(A. Necchi)*

The NEPTUNES study supports that immune checkpoint inhibitor therapy in prostate cancer should not be abandoned; instead, it could be reshaped as a combinatorial therapy in biomarker-selected patients.*

*Relevance section written by *JCO* Associate Editor Andrea Necchi, MD.

low tumor mutational burden (TMB), low neoantigen burden, and low checkpoint molecule expression.[6,7] Enrichment of T cells within the tumor microenvironment has been correlated with CPI response in a range of tumors.[8-11] In patients with mCRPC and biallelic *CDK12* loss, 2/4 patients responded, specifically those with high inflammatory infiltrate (HII).[12] We and others have shown that a subgroup of patients with prostate cancer have a prominent inflammatory infiltrate,[7,13] but whether this enriches for response to CPI is unknown. Additionally, genomic aberrations in DNA repair genes[14] have been linked to increased neoantigen burden, higher immune infiltrate, and higher response rates to CPI.[12,15-17]

We hypothesized that patients with mCRPC would be more likely to respond to combination CPI if they had immunogenic molecular or pathologic features. We present the results of the NEPTUNES phase II study, where we tested two schedules of nivolumab and ipilimumab in molecularly selected patients with mCRPC.

MATERIALS AND METHODS

Study Design and Patients

This multicenter, open-label, phase II study recruited patients in two sequential dosing cohorts testing the response, survival, and safety of the combination of ipilimumab and nivolumab in patients deemed to have a putative immunogenic molecular profile. Cohort 2 (C2) was opened after the successful recruitment to cohort 1 (C1) to test efficacy and safety of lower dose of ipilimumab and higher dose of nivolumab. The study was conducted in nine hospitals across the United Kingdom.

Patients with metastatic prostate cancer age 18 years and older, who had histologically confirmed prostate adenocarcinoma, and either had archival prostate cancer tissue available or were willing to undergo a new biopsy were eligible for prescreening (Data Supplement, online only, Trial protocol).

Patients were deemed to be immunogenic signature–positive (ImS+) if they had at least one of the following: (1) Loss of expression of mismatch repair proteins (MLH1, MSH2, MSH6, and/or PMS2) by immunohistochemistry (mismatch repair deficiency [MMRD]), (2) a deleterious biallelic aberration in one or more genes involved in the DNA repair machinery as assessed by next-generation sequencing (DNA damage repair deficiency [DDRD]), and (3) HII on a multiplexed immunohistochemistry assay defined as T cells (CD8+/CD4+/FOXP3+) representing more than 20% of the total nucleated cells in the region of interest (Data Supplement, Fig S1).

ImS+ patients could be considered for enrollment into the main study. Patients had to have WHO performance status 0-1, life expectancy ≥6 months, and confirmed progression to mCRPC, while on continuous androgen-deprivation therapy with a serum testosterone <1.73 nmol/L. Patients were also required to have progressed on at least one life-prolonging systemic therapy for prostate cancer other than androgen-deprivation therapy (ie, androgen receptor pathway inhibitor, taxane-based chemotherapy, or radium223).

The trial protocol was approved by the National Research Ethics Committee (17/SC/0369). This study adhered to the principles of the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulations. The authors guarantee the accuracy and completeness of the data and analysis, and affirm adherence to the protocol throughout the trial. This trial is registered with ClinicalTrials.gov (NCT03061539; February 15, 2017).

Procedures

Patients participating in the prescreening phase had their tumor tissue analyzed (Data Supplement). Multiplexed immunohistochemistry for CD8, CD4, and FoxP3 was carried out on the tumor samples as described previously.[7] Four slides were stained by immunohistochemistry for MLH1, MSH2, MSH6, and PMS2 using the US Food and Drug Administration (FDA)–approved VENTANA MMR RxDx Panel (Roche Diagnostics, Rotkreuz, Switzerland) as per manufacturer instructions. UW-OncoPlex comprehensive genomic profiling, which included microsatellite instability and TMB analysis, was performed at the University of Washington.[18,19]

In C1, patients received 1 mg/kg nivolumab and 3 mg/kg ipilimumab (Nivo1/Ipi3) once every three weeks for up to 4 cycles followed by a 6-week delay and then a 480-mg flat dose of nivolumab once every 4 weeks for up to 10 cycles. In C2, patients received 3 mg/kg nivolumab and 1 mg/kg ipilimumab (Nivo3/Ipi1) once every three weeks and had a 3-week delay before flat dose nivolumab. Patients received treatment until unequivocal progression, unacceptable toxicity, or withdrawal of consent. Early discontinuation of ipilimumab and start of monotherapy was allowed for patients who could not tolerate combination treatment. Dose reductions were not permitted. If treatment was delayed by >6 weeks, the patient had to permanently discontinue trial treatment. Patients could continue treatment, irrespective of prostate-specific antigen (PSA) and imaging results, provided they were deriving clinical benefit as assessed by the treating physician.

Patients underwent a CT chest abdomen and pelvis and bone scan at baseline, weeks 9, 18, and 27, then every 12 weeks up to 2 years, and every 6 months afterward up to 5 years. Baseline disease features at the time of registration were collected, including presence of measurable disease and burden of disease as defined in the CHAARTED trial.[20] Radiologic response was assessed using modified RECIST1.1 and PCWG3 criteria for CT and bone scans, respectively. A blood sample for circulating tumor cells (CTC) response assessment was collected at the same time points together with additional blood samples for exploratory biomarker analyses. CTC were enumerated using the CellSearch Platform.

Adverse events (AEs) were recorded on the basis of patients' reports, physical examination, and laboratory tests, and graded using Common Terminology Criteria for AEs, version 4.03.

Outcomes

The primary outcome measure was the composite response rate (CRR). A patient was considered to have achieved a response if any of the following criteria were met: (1) radiologic response confirmed ≥4 weeks later; (2) PSA response ≥50% confirmed by a second PSA test ≥4 weeks later; or (3) conversion of CTC count from ≥5 cells/7.5 mL at baseline to <5 cells/7.5 mL at 9 weeks confirmed by a second CTC test ≥4 weeks later as previously described.[21]

The secondary end points included overall survival (OS), progression-free survival, duration of response, and an assessment of the frequency and severity of AEs. OS was measured as the time from the date of registration to the date of death from any cause. Patients were censored at the date last seen alive if they did not have a reported death at the time of final analysis. Progression-free survival time was measured as the time from registration to either objective radiologic progression (RECIST 1.1), PSA progression (PCWG3), unequivocal evidence of clinical progression, or death from any cause, whichever occurred first. Patients without documented progression or death were censored at the date of the last follow-up. Duration of response was defined as the time from composite response to progression or death.

An exploratory analysis was conducted to assess the disease control rate at 6 months (DCR6), defined as absence of a progression event (as per same definition used for progression free survival) beyond 6 months from registration. Subgroup analyses on the basis of baseline characteristics were conducted in the combined cohort population.

Statistical Analysis

The study was designed so that each cohort would be analyzed separately. The sample size for both arms was calculated using an A'Hern single-stage Phase II design, where the null hypothesis was a CRR of 20% or below, while the alternative hypothesis was a CRR of 40% (with a one-sided α level of 0.05 and a β level of 0.20). Following the A'Hern design, if at least 12 (34%) of 35 evaluable patients had a treatment composite response (PSA, CTC, or radiologic), then the null hypothesis would be rejected. Comparisons for continuous and categorical variables were performed using Wilcoxon rank sum test and chi-square test, respectively. A Cox proportional-hazards model was used to compare duration of response and survival across cohorts. Time-to-event outcomes were reported using standard survival analysis on the basis of Kaplan-Meier method. AEs were reported descriptively using frequencies and percentages.

RESULTS

Between February 2018 and April 2022, 380 patients were enrolled into prescreening. The overall rate of ImS+ samples

was 31% (n = 119) with 11% (n = 41) of the samples failing the test, most commonly because of insufficient tumor DNA (Data Supplement, Fig S2). Overall, 74 ImS+ patients (recruitment target was originally 71) were enrolled into the main study. C1 recruited 36 patients between May 2018 and October 2020, and C2 recruited 38 patients between November 2020 and June 2022. One patient in C1 and two patients in C2 withdrew from the study before drug dosing. These three patients were replaced and not included in efficacy analysis. Patients were deemed ImS+ as per the following features: 10/71 (14%) had MMRD, 48/71 (68%) had DDRD, and 30/71 (42%) had HII, with 14/71 (20%) meeting multiple criteria (Fig 1).

The median age was 67 years (range, 50-78) in C1 and 70 years (range, 53-82) in C2, with a performance status of 0 in 47% and 45% patients, respectively. Patients were heavily pretreated with 50% and 60% patients having had ≥3 previous life-prolonging therapies, including 89% and 71% having received docetaxel, in C1 and C2, respectively. Measurable disease was detected at baseline in 53% and 50% of patients, with 67% and 61% having high burden of disease at the time of registration in C1 and C2, respectively (Table 1, Data Supplement, Table S1).

The CRR was 14/35 (40%) patients in C1 enabling rejection of the null hypothesis. In C2, the CRR of 9/36 (25%) patients was insufficient to reject the null hypothesis. Overall, CRR in both cohorts was 23/71 (32%, 90% CI, 23 to 43), significantly higher than the prespecified threshold for an ineffective treatment at ≤20% (Table 2). CRR was higher in patients with RECIST-measurable disease (51%, 90% CI, 37% to 66%) than with non–RECIST-measurable disease (12%, 90% CI, 4% to 25%). In both cohorts, all patients who had a radiologic response also had a PSA response (Data Supplement, Fig S3), hence the difference observed is not due to the absence of radiologic responses in the patients with non–RECIST-measurable disease. Patients also had a higher CRR if they had received previous docetaxel treatment (38% [90% CI, 27% to 49%] *v* 13% [90% CI, 2% to 36%], Data Supplement, Table S2).

CTC response could not be assessed for 22/35 (63%) and 21/36 (58%) patients in C1 and C2, respectively, the main reason being insufficient CTCs (<5 cells/7.5 mL) at baseline to observe a conversion at week 9. Of the seven patients with a CTC response, only two patients had a CTC-only response.

Responses were recorded for 7/10 (70%) patients with MMRD, 4/8 (50%) patients with biallelic BRCA2 loss-of-function, and 2/7 (29%) patients with biallelic CDK12 loss-of-function. CRR in patients with exclusively ATM, CHD1, or CHEK2 biallelic alterations was low (2/23, 9%). Of the 20 patients who were enrolled into the main study on the basis of HII alone, seven (35%) responded to treatment. The CRR in all patients who had HII, regardless of genomic features, was 43% (13/30).

TMB was evaluable in 64/71 patients (90%; responders n = 21, nonresponders n = 43) across both cohorts. TMB was higher among responders versus nonresponders (median responders, 4 mut/Mb [IQR, 1.5-15.5] *v* nonresponders, 2 mut/Mb [IQR, 2-3], $P = .007$, Wilcoxon test). However, the difference was primarily driven by enrichment for MMRD patients among the responders (median MMRD 18.5 mut/Mb [IQR, 13.5-23] *v* MMR proficient 2 mut/Mb [IQR, 1-3]). No difference in the TMB between responders and nonresponders was observed after excluding MMRD patients (Data Supplement, Fig S4).

With a follow-up time of 50.8 months in C1 and 30.6 months in C2, the median progression-free survival time was 6.6 months (95% CI, 4.9 to 10.0) in C1 and 4.0 months (95% CI, 3.2 to 7.6) in C2 (Fig 2A). Median OS time was 16.2 months (95% CI, 9.2 to 22.8) in C1 and 16.6 months (95% CI, 10.7 to 33.4) in C2 (Fig 2B).

The median duration of response was 10.2 months (95% CI, 1.8 to 17.4) in C1 and 6.4 months (95% CI, 1.1 to NR) in C2. Duration of response was significantly longer in patients with MMRD (10.2 months) or DDRD (17.4 months) compared with patients with only HII (2.6 months—Data Supplement, Fig S5). Median duration of response for HII ± other features was 6.5 months.

The DCR6 was 57% in C1 and 44% in C2. Overall, 36/71 (51%) patients had DCR6. Of the 23 patients achieving a composite response, 15 also had a duration of response >6 months.

The use of steroid was common in the NEPTUNES trial, with 37/71 (52%) patients receiving steroids during treatment or follow-up period. Specifically, 34/37 patients received high-dose steroids (prednisolone >10 mg or equivalent dose) during trial treatment. For one additional patient, the dose was not specified. Among the 12 responders who received steroids, only four had steroids administered before first evidence of response, six started steroids after response had already been confirmed, while for two patients, the date of initiation was missing.

All treated patients were evaluable for toxicity. Treatment-related AEs (TRAEs) of any grade occurred in 34/35 patients in C1 and 28/36 patients in C2. Grade 3 to 4 TRAEs occurred in 22/35 (63%) patients in C1 and 12/36 (33%) patients in C2 (Table 3 and Data Supplement, Table S3). There were no treatment-related deaths. At least one serious AE was recorded for 49/71 patients, with 31/71 having a serious TRAE (Data Supplement, Table S4). In C1, there were 17 (49%) patients who required a dose delay and 12 (34%) who had to permanently discontinue treatment because of toxicities. For C2, this was 13 (36%) and 6 (17%), respectively (Data Supplement).

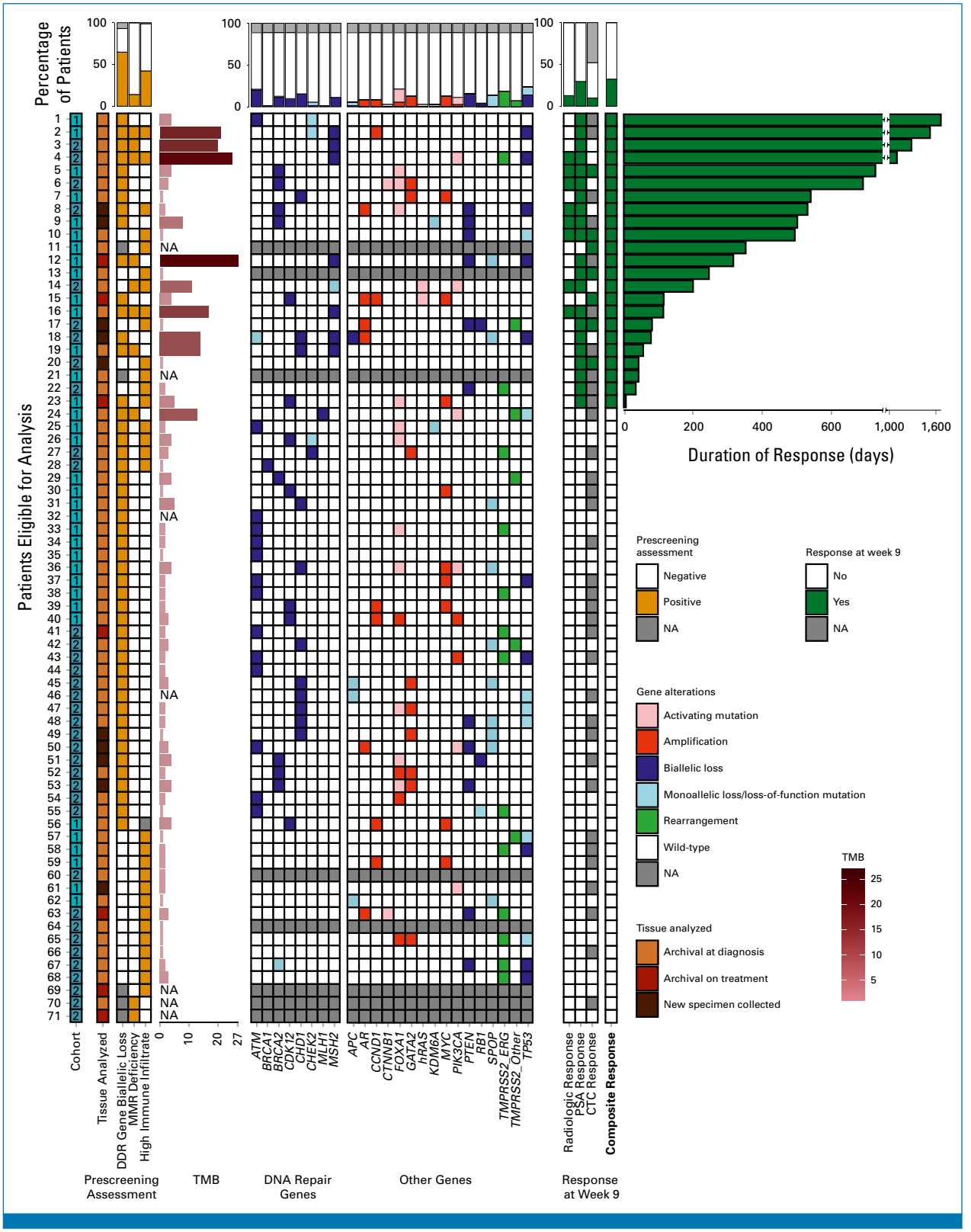

FIG 1. Patient response by molecular selection criteria. TMB and gene alterations determined using the University of Washington OncoPlex assay. Different types of gene alterations are defined by color code. Only genes that were altered in ≥2 patients are included. TMB and gene alterations were deemed NA (gray boxes) when the OncoPlex assay was not performed (because of insufficient sample quality/quantity) or results were uncertain because of low tumor content. Stacked bars above feature columns represent proportion of patients who are positive/negative for that feature. Duration of response (right) is represented using a discontinuous *x*-axis to include four patients with response >4 years. CTC, circulating tumor cells; DDR, DNA damage repair; MMR, mismatch repair; NA, data not available; PSA, prostate-specific antigen; TMB, tumor mutational burden.

TABLE 1. Baseline Characteristics

Baseline Characteristic	Cohort 1 (n = 36)	Cohort 2 (n = 38)
Age, years		
Median (range)	67 (50-78)	70 (53-82)
WHO PS, No. (%)		
Fully active	17 (47)	17 (45)
Restricted in physical activity	19 (53)	21 (55)
Baseline PSA (ng/mL)		
Median (range)	88.5 (0.4-13,610.4)	140.7 (2.7-3,107.7)
Previous docetaxel, No. (%)		
No	4 (11)	11 (29)
Yes	32 (89)	27 (71)
Previous ARPI, No. (%)		
No	5 (14)	1 (3)
Yes	31 (86)	37 (97)
No. of previous therapies, No. (%)		
1	4 (11)	5 (13)
2	14 (39)	10 (26)
3	11 (31)	16 (42)
≥4	7 (19)	7 (18)
Biomarker features, No. (%)		
MMRD by IHC	5 (14)	5 (13)
MMRD and homologous recombination deficiency	1 (3)	0 (0)
MMRD with unknown homologous recombination status	0 (0)	2 (5)
MMRD and HII	2 (6)	2 (5)
MMRD only	2 (6)	1 (3)
Homologous recombination deficiency	23 (64)	21 (55)
BRCA	3 (8)	7 (18)
Non-BRCA	20 (56)	14 (37)
HII	14 (39)	17 (45)
HII and MMRD	2 (6)	2 (5)
HII and homologous recombination deficiency	3 (8)	3 (8)
HII with unknown homologous recombination status	3 (8)	3 (8)
HII only	6 (17)	9 (24)

Abbreviations: ARPI, androgen receptor pathway inhibitor; HII, high inflammatory infiltrate; IHC, immunohistochemistry; MMRD, mismatch repair deficiency; PS, performance status; PSA, prostate-specific antigen.

TABLE 2. Composite Response by Cohort

	All Patients		Cohort 1		Cohort 2	
	N= 71		n = 35		n = 36	
Response	No. (%)	90% CI	No. (%)	90% CI	No. (%)	90% CI
Composite response rate	23 (32)	23% to 43%	14 (40)	26% to 55%	9 (25)	14% to 40%
PSA response	21 (30)	21% to 40%	12 (34)	21% to 50%	9 (25)	14% to 40%
Radiologic response	8 (11)	6% to 19%	4 (11)	4% to 24%	4 (11)	4% to 24%
CTC response	7 (10)	5% to 18%	5 (14)	6% to 28%	2 (6)	1% to 16%

Abbreviations: CTC, circulating tumor cells; PSA, prostate-specific antigen.

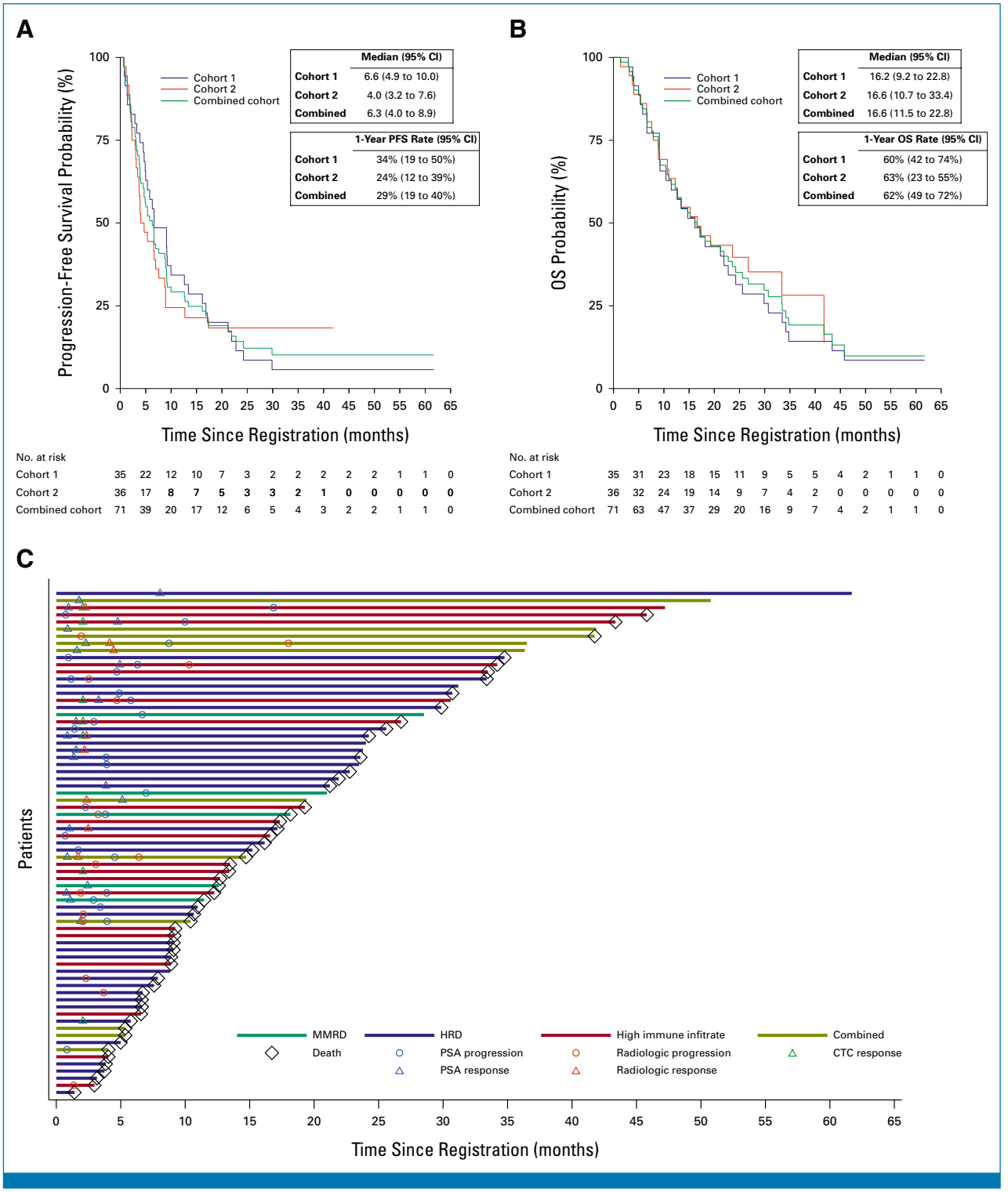

FIG 2. Patient survival and types of response. (A) progression-free survival; (B) OS; and (C) Swimmer plot showing clinical course of individual patients including time and type of response and progression on trial treatment. CTC, circulating tumor cells; HRD, homologous recombination deficiency; MMRD, mismatch repair deficiency; OS, overall survival; PSA, prostate-specific antigen.

DISCUSSION

NEPTUNES met its primary end point demonstrating a CRR of 40% in C1 and 32% in the combined cohort. To our knowledge, this is the first study in prostate cancer to prospectively enroll patients on the basis of a signature including inflammatory infiltrate. The CRR of 43% in patients with HII is a remarkable increase compared with what

TABLE 3. Treatment-Related AE by Cohorts

	Cohort 1		Cohort 2		Combined Cohort	
Organ Class and AE Name (CTCAE v4.03)[a]	Any Grade, No. (%)	Grade 3 to 4, No. (%)	Any Grade, No. (%)	Grade 3 to 4, No. (%)	Any Grade, No. (%)	Grade 3 to 4, No. (%)
Blood and lymphatic system disorders						
Anemia	3 (8.6)	2 (5.7)	1 (2.8)	—	4 (5.6)	2 (2.8)
Endocrine disorders						
Adrenal insufficiency	3 (8.6)	2 (5.7)	3 (8.3)	1 (2.8)	6 (8.5)	3 (4.2)
Hyperthyroidism	7 (20.0)	—	—	—	10 (14.1)	—
Hypothyroidism	4 (11.4)	—	8 (22.2)	1 (2.8)	12 (16.9)	1 (1.4)
Eye disorders						
Retinal vascular disorder	1 (2.9)	1 (2.9)	—	—	1 (1.4)	1 (1.4)
Uveitis	1 (2.9)	1 (2.9)	—	—	1 (1.4)	1 (1.4)
GI disorders						
Colitis	8 (22.9)	5 (14.3)	2 (5.6)	1 (2.8)	10 (14.1)	6 (8.5)
Diarrhea	23 (65.7)	7 (20.0)	15 (41.7)	3 (8.3)	38 (53.5)	10 (14.1)
Nausea	13 (37.1)	1 (2.9)	6 (16.7)	1 (2.8)	19 (26.8)	2 (2.8)
Vomiting	8 (22.9)	1 (2.9)	4 (11.1)	1 (2.8)	12 (16.9)	2 (2.8)
General disorders and administration site conditions						
Fatigue	14 (40.0)	5 (14.3)	10 (27.8)	—	24 (33.8)	5 (7.0)
Fever	7 (20.0)	—	—	—	10 (14.1)	—
Infusion-related reaction	—	—	1 (2.8)	1 (2.8)	1 (1.4)	1 (1.4)
Immune system disorders						
Other: hepatitis	1 (2.9)	—	1 (2.8)	1 (2.8)	2 (2.8)	1 (1.4)
Other: hypophysitis	2 (5.7)	1 (2.9)	—	—	2 (2.8)	1 (1.4)
Other: myasthenia gravis	—	—	1 (2.8)	1 (2.8)	1 (1.4)	1 (1.4)
Infections and infestations						
Anorectal infection	1 (2.9)	1 (2.9)	—	—	1 (1.4)	1 (1.4)
Investigations						
Alanine aminotransferase increased	5 (14.3)	1 (2.9)	3 (8.3)	2 (5.6)	8 (11.3)	3 (4.2)
Alkaline phosphatase increased	—	—	1 (2.8)	1 (2.8)	1 (1.4)	1 (1.4)
Aspartate aminotransferase increased	4 (11.4)	1 (2.9)	4 (11.1)	2 (5.6)	8 (11.3)	3 (4.2)
Serum amylase increased	4 (11.4)	1 (2.9)	1 (2.8)	1 (2.8)	5 (7.0)	2 (2.8)
Weight loss	4 (11.4)	—	4 (11.1)	—	8 (11.3)	—
Metabolism and nutrition disorders						
Acidosis	—	—	1 (2.8)	1 (2.8)	1 (1.4)	1 (1.4)
Anorexia	6 (17.1)	—	4 (11.1)	1 (2.8)	10 (14.1)	1 (1.4)
Hyperglycemia	2 (5.7)	1 (2.9)	3 (8.3)	1 (2.8)	5 (7.0)	2 (2.8)
Hypokalemia	2 (5.7)	1 (2.9)	—	—	2 (2.8)	1 (1.4)
Hyponatremia	2 (5.7)	1 (2.9)	1 (2.8)	1 (2.8)	3 (4.2)	2 (2.8)
Musculoskeletal and connective tissue disorders						
Arthralgia	5 (14.3)	1 (2.9)	2 (5.6)	1 (2.8)	7 (9.9)	2 (2.8)
Back pain	—	—	1 (2.8)	1 (2.8)	1 (1.4)	1 (1.4)
Myalgia	5 (14.3)	—	—	—	—	—
Nervous system disorders						
Headache	5 (14.3)	—	—	—	—	—
Lethargy	5 (14.3)	—	4 (11.1)	—	9 (12.7)	—
Renal and urinary disorders						
Acute kidney injury	4 (11.4)	—	—	—	—	—
Other: nephritis	—	—	1 (2.8)	1 (2.8)	1 (1.4)	1 (1.4)

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TABLE 3. Treatment-Related AE by Cohorts (continued)

Organ Class and AE Name (CTCAE v4.03)[a]	Cohort 1		Cohort 2		Combined Cohort	
	Any Grade, No. (%)	Grade 3 to 4, No. (%)	Any Grade, No. (%)	Grade 3 to 4, No. (%)	Any Grade, No. (%)	Grade 3 to 4, No. (%)
Respiratory, thoracic, and mediastinal disorders						
Dyspnea	7 (20.0)	—	3 (8.3)	1 (2.8)	10 (14.1)	1 (1.4)
Pneumonitis	7 (20.0)	2 (5.7)	3 (8.3)	—	10 (14.1)	2 (2.8)
Skin and subcutaneous tissue disorders						
Erythema	1 (2.9)	1 (2.9)	—	—	1 (1.4)	1 (1.4)
Other: rash unspecified	11 (31.4)	2 (5.7)	2 (5.6)	—	13 (18.3)	2 (2.8)
Pruritus	9 (25.7)	—	6 (16.7)	—	15 (21.1)	—
Rash maculopapular	12 (34.3)	3 (8.6)	2 (5.6)	—	14 (19.7)	3 (4.2)
Vascular disorders						
Vasculitis	1 (2.9)	1 (2.9)	—	—	1 (1.4)	1 (1.4)

Abbreviations: AEs, adverse events; CTCAE, Common Terminology Criteria for Adverse Events.
[a]AEs by organ system included in the table if at least one grade 3 to 4 event was recorded or incidence of any grade more than 10%.

is observed in unselected patients.[2,5] Notably, only 40% of MMRD tumors exhibited HII, indicating that the evaluation of multiple biomarkers is crucial to identify patients with mCRPC who could respond to CPI. A small phase II trial recently recruited patients with early-stage triple-negative breast cancer with high tumor-infiltrating lymphocytes to receive neoadjuvant nivolumab and ipilimumab, reporting a pathologic complete response rate of 33% (5/15 patients), and larger trials using this patient selection are in accrual.[22]

NEPTUNES results corroborate the recently published data from the INSPIRE study. This study recruited patients with mCRPC and MMRD, TMB ≥7.1 muts/Mb, presence of biallelic *CDK12* inactivation, or *BRCA2* mutation to receive Nivo3/Ipi1 followed by nivolumab maintenance, creating some overlap with the NEPTUNES trial. Both trials reported excellent outcome for patients with MMRD.[23] Interestingly, in the retrospective series from Graham et al and Sena et al,[24,25] a lower proportion of patients with MMRD, 8/15 (53%) and 11/17 (65%), respectively, achieved a biochemical response to CPI monotherapy. In the prospective MMRD pan-tumor Keynote-158 study, 2/8 (25%) had a radiologic response.[26] Collectively, these data suggest that combination CPI should be tested against monotherapy in prospective clinical trials.

Response in the *BRCA2*-aberrant population was 23% in the INSPIRE trial versus 50% in this study. Multiple factors may have contributed to this difference, including the smaller patient subgroup in NEPTUNES (8 *v* 20); the higher dose of ipilimumab administered in C1; and the inclusion of patients with monoallelic *BRCA2* mutations and/or BRCAness signature in INSPIRE versus the more-strict NEPTUNES criteria, which only allowed patients with *BRCA2* biallelic loss.

We did not include TMB as a selection biomarker in the NEPTUNES study as a clear threshold for TMB had not been defined in prostate cancer at the time of study design. Although TMB did correlate with response in our study, the correlation was lost when MMRD patients were excluded from the analysis. Both the CheckMate 650 and the INSPIRE trials did not show a consistent association between efficacy and TMB.[23,27]

CTC data were lacking, mainly because of insufficient cells detected at baseline. Only two patients had a CTC-only response, and the duration of response was short. Therefore, CTC enumeration seems to have limited utility in the response assessment of patients with mCRPC treated with CPIs.

Two different dose regimens of nivolumab and ipilimumab were tested. This study was not powered to detect differences in efficacy between the two regimens. With this limitation, the numerically lower CRR in the Nivo3/Ipi1 cohort did not affect OS. However, the Nivo3/Ipi1 dose regimen was considerably better tolerated (≥G3 tox, Nivo1/Ipi3 63% *v* Nivo3/Ipi1 33%) and had a lower discontinuation rate, in keeping with data from other tumor types.[28,29]

The majority of patients in this study were extensively pretreated. Unexpectedly, docetaxel-naïve patients had a numerically lower CRR compared with previously exposed patients. Notably, both the CheckMate 650 study and the INSPIRE study reported increased response rates in docetaxel-naïve patients.[5,23] CRRs were higher in patients with measurable disease. This is consistent with other trials where patients with bone-predominant disease rarely showed a PSA response after immunotherapy,[2] possibly because of a hostile immune microenvironment enriched in M2-macrophages, T-helper-17 cells, and cytokines such as TGFβ and IL-6.[30,31]

Analyses within this trial were limited by lack of contemporaneous tissue, meaning that molecular classifications often predates the CPI treatment by many years. Evaluation

of composite response was hindered by missing CTC data and the bone-predominant nature of prostate cancer metastases, and was therefore mainly driven by biochemical response. Because of the relatively small number of patients enrolled and different length of follow-up between cohorts, the subgroup analyses were not statistically powered and should be considered hypothesis-generating.

In summary, nivolumab and ipilimumab demonstrated antitumor activity in selected patients with pretreated prostate cancer. The Nivo3/Ipi1 dose schedule was better tolerated than the Nivo1/Ipi3 dose schedule and may be equally effective in prolonging patients' survival. We prospectively tested a composite biomarker that enriched for responders and has the potential to push the risk-benefit dial in favor of combination CPI in mCRPC. Over 40% of patients with HII responded to combination CPIs, regardless of the presence of other biomarkers. Larger randomized clinical studies for biomarker-selected metastatic prostate cancer are warranted.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Nivolumab and Ipilimumab for Metastatic Castration-Resistant Prostate Cancer With an Immunogenic Signature: The Multicenter, Two-Cohort, Phase II NEPTUNES Study

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NEPTUNES DATA SUPPLEMENT

Contents

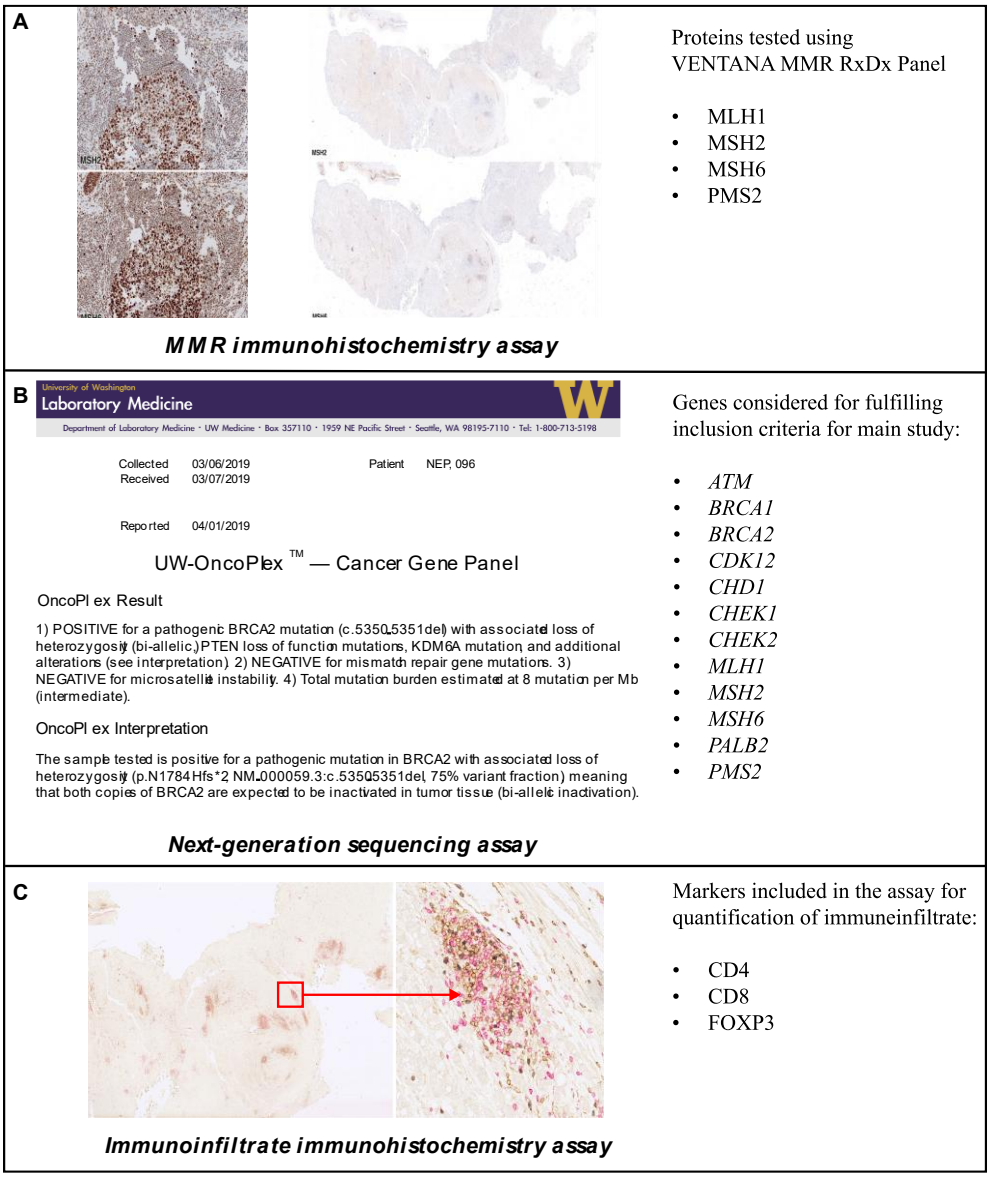

Figure S1: Immunogenic signature assays

A) Positive tissue staining for MSH2 and MSH6 as part of mismatch repair immunochemistry assay; B) OncoPlex assay report for NEP096 tissue sample, harbouring BRCA2 bi-allelic loss. C) Immunochemistry staining of T Cells within tumour tissue: brown staining represent CD4+ T Cells, red staining represent CD8+ T Cells, and blue staining represent FOXP3+ T Cells.

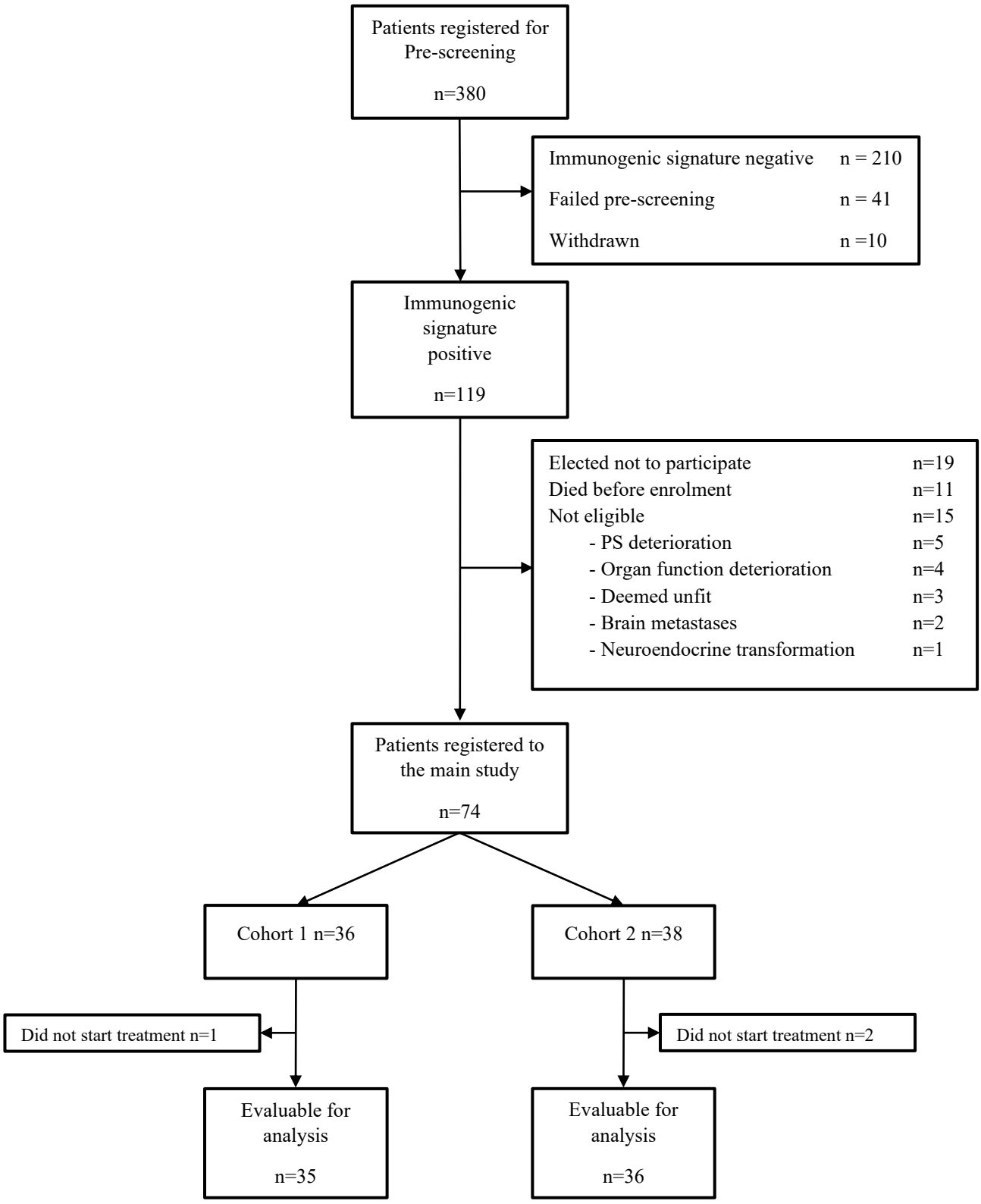

Figure S2: Trial CONSORT diagram

Table S1 - Additional patient characteristics

Baseline characteristics	Cohort 1 (%) N=36	Cohort 2 (%) N=38
BMI		
Median (range)	26.8 (19.7 to 39.5)	26.7 (21.1 to 48.7)
Bone metastatic burden		
Missing	4 (11%)	3 (8%)
Low Burden	8 (22%)	12 (32%)
High Burden	24 (67%)	23 (61%)
Measurable disease at baseline		
Measurable disease	19 (53%)	19 (50%)
Non-measurable disease	17 (47%)	19 (50%)
Previous Cabazitaxel		
Yes	15 (42%)	16 (42%)
No	21 (58%)	22 (58%)
Previous Radium 223		
Yes	3 (8%)	3 (8%)
No	33 (92%)	35 (92%)
Previous PARP inhibitor		
Yes	0 (0%)	3 (8%)
No	36 (100%)	35 (92%)
Previous radiotherapy		
Unknown	1 (3%)	4 (11%)
Yes	8 (22%)	14 (37%)
No	27 (75%)	20 (53%)

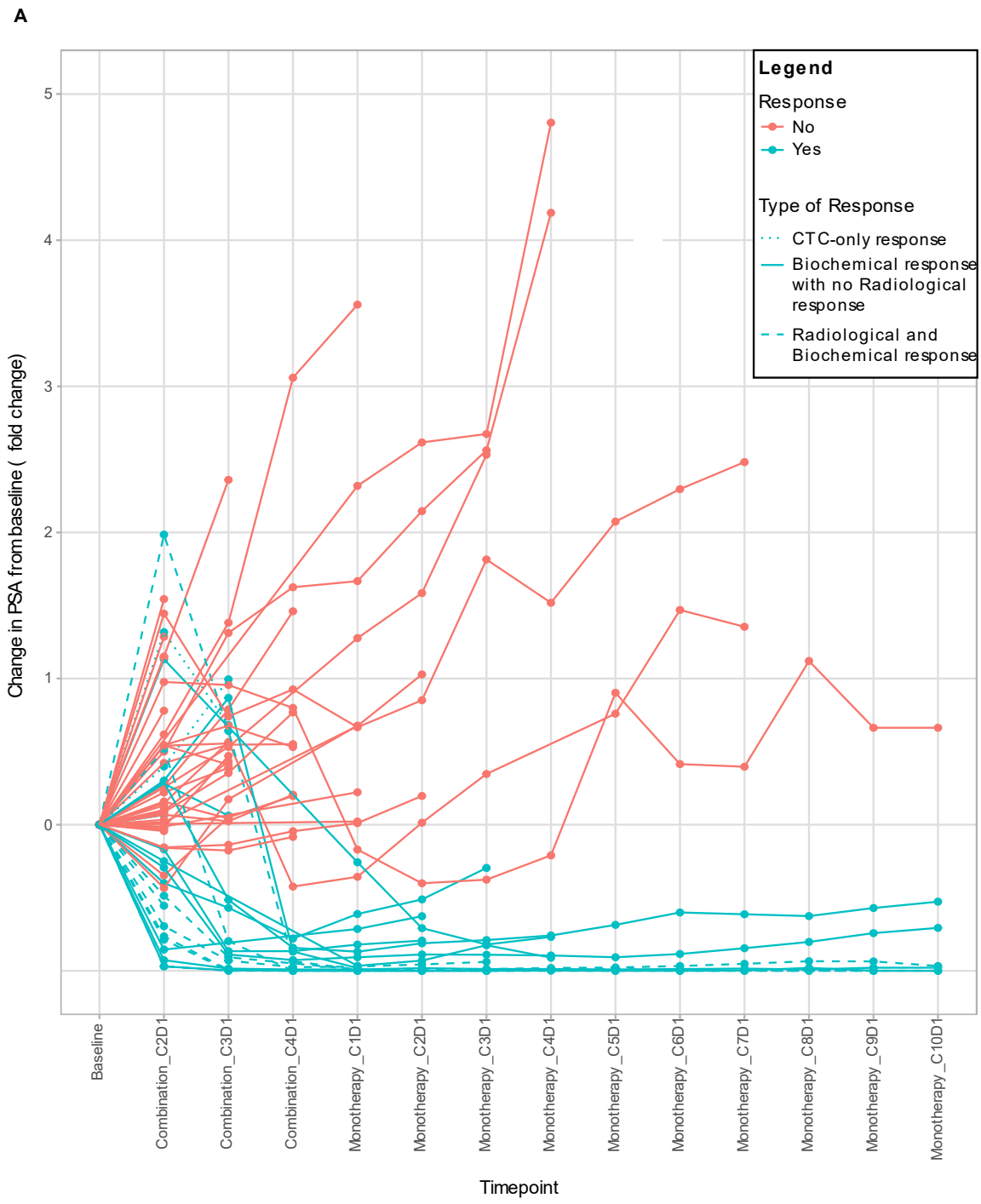

Figure S3: **PSA changes during treatment**

PSA changes from baseline. Solid lines include patients with biochemical response and CTC response but not radiological response. PSA values with fold changes from baseline >5 are not represented.

Table S2 – Response rates for patients subgroups

Baseline characteristics	Composite Response		
	Total patients	Responders (%)	90 % CI
Measurable disease			
No	34	4 (12%)	4% to 25%
Yes	37	19 (51%)	37% to 66%
Previous Docetaxel			
No	15	2 (13%)	2% to 36%
Yes	56	21 (38%)	27% to 49%
Previous radiotherapy			
No	47	14 (30%)	19% to 43%
Yes	22	8 (36%)	20% to 56%

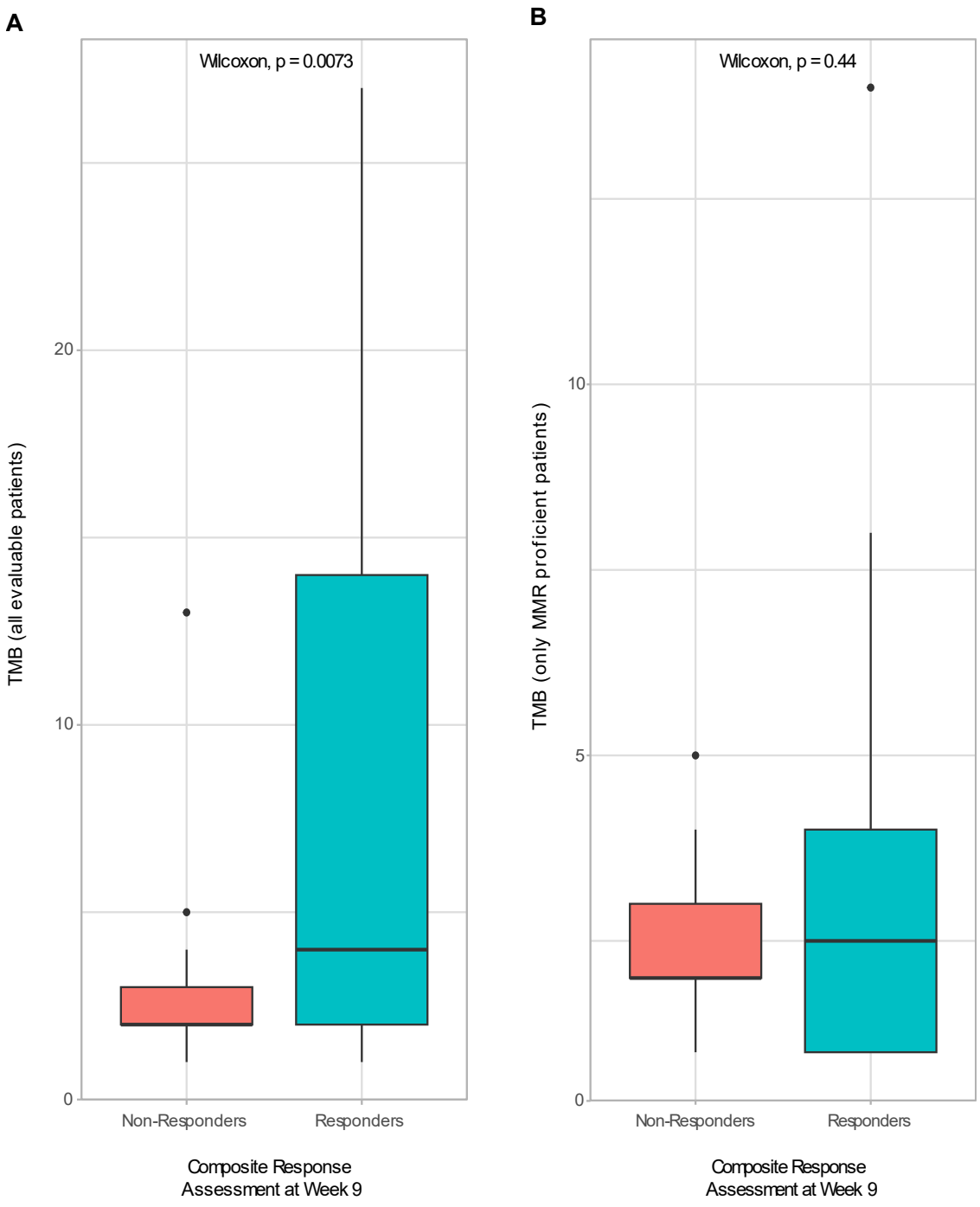

Figure S4: **Tumour mutational burden**

Distribution of tumour mutational burden across Non-Responders and Responders. A) All 71 evaluable patients included; B) Only mismatch repair proficient patients included. TMB, Tumour Mutation Burden; MMR, Mismatch Repair.

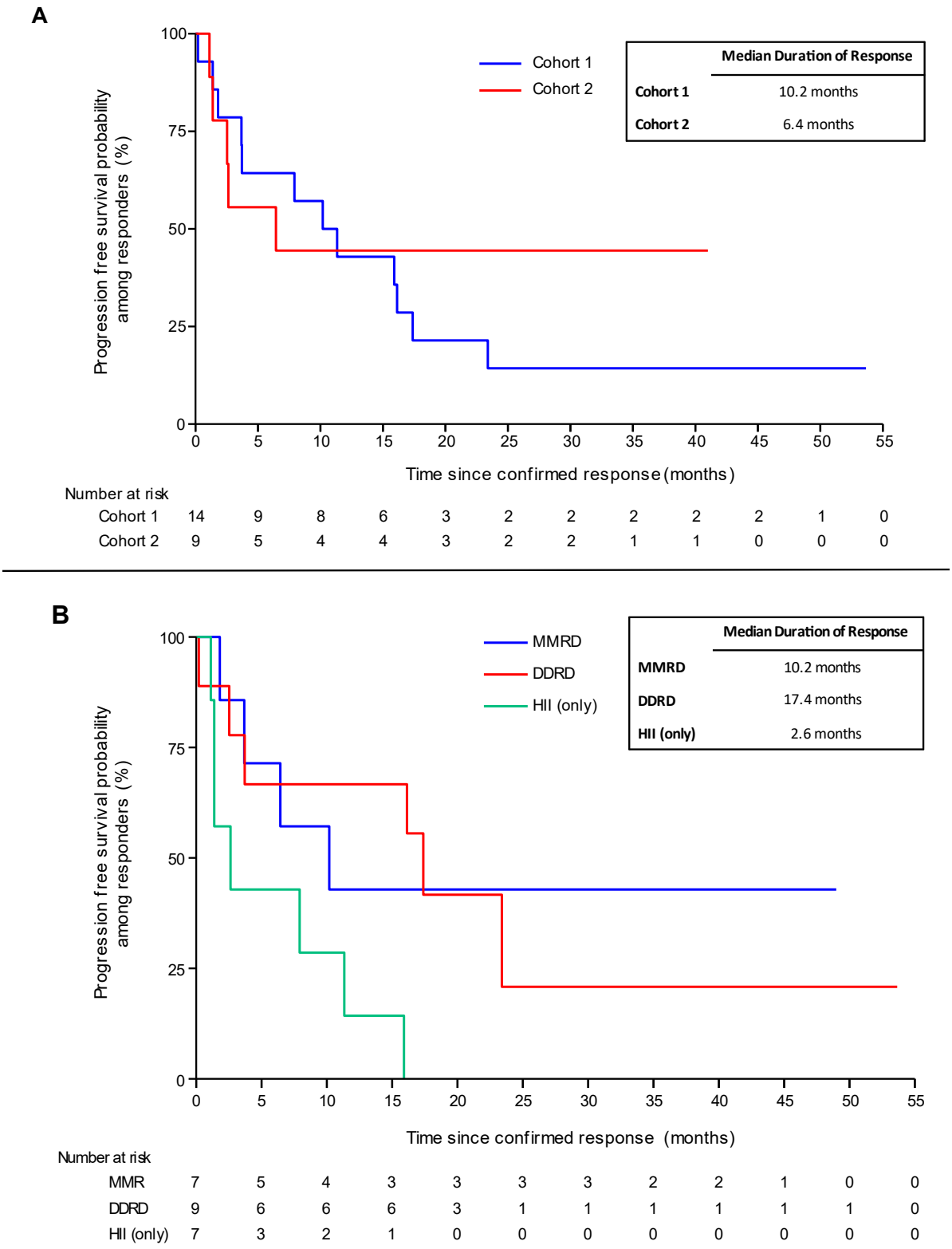

Figure S5: **Duration of response**

Duration of response categorised by A) assigned trial cohort; B) immunogenic signature component. Patients that were positive for both MMRD or DDRD and high immune infiltrate (HII) were included in the MMRD or DDRD group respectively. The HII group is exclusively high inflammatory infiltrate with no MMRD or DDRD. MMRD versus HII: HR 3.9 (95% CI: 1.0 to 14.4, p-value = 0.044); DDRD versus HII: HR 0.30 (95% CI: 0.09 to 0.96, p-value = 0.043). DDRD versus MMRD: HR 1.1 (95% CI: 0.3 to 4.1, p-value = 0.837). MMRD, mismatch repair deficiency; DDRD, DNA damage repair deficiency.

Table S3: Adverse events in treated patients

Organ class and AE names (CTCAE V4.03)	Cohort 1 Grade 1, N (%)	Cohort 1 Grade 2, N (%)	Cohort 1 Grade 3, N (%)	Cohort 1 Grade 4, N (%)	Cohort 2 Grade 1, N (%)	Cohort 2 Grade 2, N (%)	Cohort 2 Grade 3, N (%)	Cohort 2 Grade 4, N (%)
Blood and lymphatic system disorders								
Anemia	-	1 (2.9%)	2 (5.7%)	-	1 (2.8%)	-	-	-
Cardiac disorders								
Atrial fibrillation	-	-	-	-	-	1 (2.8%)	-	-
Chest pain: cardiac	1 (2.9%)	-	-	-	-	-	-	-
Palpitations	-	-	-	.	1 (2.8%)	-	-	-
Pericardial effusion	-	1 (2.9%)	-	-	-	-	-	-
Ear and labyrinth disorders								
Vestibular Disorder	1 (2.9%)	-	-	-	-	-	-	-
Endocrine disorders								
Adrenal insufficiency	-	1 (2.9%)	2 (5.7%)	-	1 (2.8%)	1 (2.8%)	1 (2.8%)	-
Hyperthyroidism	5 (14.3%)	2 (5.7%)	-	-	1 (2.8%)	2 (5.6%)	-	-
Hypothyroidism	2 (5.7%)	2 (5.7%)	-	-	2 (5.6%)	5 (13.9%)	1 (2.8%)	-
Other : Hypopituitarism	-	2 (5.7%)	-	-	-	1 (2.8%)	-	-
Eye disorders								
Blurred vision	-	1 (2.9%)	-	-	-	-	-	-
Conjunctivitis	-	1 (2.9%)	-	-	-	-	-	-
Retinal Vascular Disorder	-	-	1 (2.9%)	-	-	-	-	-
Uveitis	-	-	-	1 (2.9%)	-	-	-	-
Watering eyes	1 (2.9%)	-	-	-	-	-	-	-
Gastrointestinal disorders								
Abdominal pain	2 (5.7%)	-	-	-	1 (2.8%)	-	-	-
Colitis	-	3 (8.6%)	5 (14.3%)	-	1 (2.8%)	-	1 (2.8%)	-
Constipation	1 (2.9%)	-	-	-	1 (2.8%)	-	-	-
Diarrhea	9 (25.7%)	7 (20.0%)	7 (20.0%)	-	9 (25.0%)	3 (8.3%)	3 (8.3%)	-
Dry mouth	1 (2.9%)	-	-	-	1 (2.8%)	1 (2.8%)	-	-
Gastritis	-	1 (2.9%)	-	-	-	-	-	-
Gastroesophageal reflux disease	-	-	-	-	-	1 (2.8%)	-	-
Mucositis oral	2 (5.7%)	-	-	-	1 (2.8%)	1 (2.8%)	-	-
Nausea	8 (22.9%)	4 (11.4%)	1 (2.9%)	-	3 (8.3%)	2 (5.6%)	1 (2.8%)	-
Oral dysesthesia	1 (2.9%)	-	-	-	-	-	-	.
Oral pain	1 (2.9%)	-	-	-	-	-	-	-
Other : Mucus In Stool	-	-	-	-	1 (2.8%)	-	-	-
Other: Loose stool	1 (2.9%)	-	-	-	-	-	-	-
Stomach pain	1 (2.9%)	-	-	-	-	-	-	-
Vomiting	2 (5.7%)	5 (14.3%)	1 (2.9%)	-	3 (8.3%)	-	1 (2.8%)	-
General disorders and administration site conditions								
Edema Limbs	1 (2.9%)	-	-	-	-	1 (2.8%)	-	-
Fatigue	5 (14.3%)	4 (11.4%)	5 (14.3%)	-	10 (27.8%)	-	-	-
Fever	6 (17.1%)	1 (2.9%)	-	-	3 (8.3%)	-	-	-
Flu like symptoms	-	-	-	-	-	1 (2.8%)	-	-
Infusion related reaction	-	-	-	-	-	-	1 (2.8%)	-

Organ class and AE names (CTCAE V4.03)	Cohort 1 Grade 1, N (%)	Cohort 1 Grade 2, N (%)	Cohort 1 Grade 3, N (%)	Cohort 1 Grade 4, N (%)	Cohort 2 Grade 1, N (%)	Cohort 2 Grade 2, N (%)	Cohort 2 Grade 3, N (%)	Cohort 2 Grade 4, N (%)
Immune system disorders								
Other: Grave's Disease	-	-	-	-	-	1 (2.8%)	-	-
Other: Hepatitis	-	1 (2.9%)	-	-	-	-	1 (2.8%)	-
Other: Hypophysitis	-	1 (2.9%)	1 (2.9%)	-	-	-	-	-
Other: Myastenia Gravis	-	-	-	-	-	-	1 (2.8%)	-
Other: Thyroiditis	1 (2.9%)	1 (2.9%)	-	-	2 (5.6%)	1 (2.8%)	-	-
Infections and infestations								
Anorectal Infection	-	-	1 (2.9%)	-	-	-	-	-
Bronchial infection	-	1 (2.9%)	-	-	-	-	-	-
Lung infection	-	2 (5.7%)	-	-	-	-	-	-
Mucosal infection	-	-	-	-	1 (2.8%)	-	-	-
Rash pustular	1 (2.9%)	-	-	-	-	-	-	-
Skin infection	-	1 (2.9%)	-	-	-	-	-	-
Injury, poisoning and procedural complications								
Stomal ulcer	-	1 (2.9%)	-	-	-	-	-	-
Investigations								
Alanine aminotransferase increased	4 (11.4%)	-	1 (2.9%)	-	1 (2.8%)	-	2 (5.6%)	-
Alkaline phosphatase increased	-	-	-	-	-	-	1 (2.8%)	-
Aspartate aminotransferase increased	2 (5.7%)	1 (2.9%)	1 (2.9%)	-	1 (2.8%)	1 (2.8%)	2 (5.6%)	-
Blood bilirubin increased	-	-	-	-	1 (2.8%)	-	-	-
GGT increased	1 (2.9%)	1 (2.9%)	-	-	1 (2.8%)	-	-	-
Lipase increased	2 (5.7%)	-	-	-	-	-	-	-
Other: White Blood Cells Increased	1 (2.9%)	-	-	-	-	-	-	-
Serum amylase increased	2 (5.7%)	1 (2.9%)	-	1 (2.9%)	-	-	1 (2.8%)	-
Weight loss	4 (11.4%)	-	-	-	1 (2.8%)	3 (8.3%)	-	-
Metabolism and nutrition disorders								
Acidosis	-	-	-	-	-	-	1 (2.8%)	-
Anorexia	3 (8.6%)	3 (8.6%)	-	-	2 (5.6%)	1 (2.8%)	1 (2.8%)	-
Dehydration	-	1 (2.9%)	-	-	-	-	-	-
Hypercalcemia	-	-	-	-	1 (2.8%)	-	-	-
Hyperglycemia	1 (2.9%)	-	-	1 (2.9%)	2 (5.6%)	-	-	1 (2.8%)
Hypoalbuminemia	-	-	-	-	-	1 (2.8%)	-	-
Hypokalemia	1 (2.9%)	-	1 (2.9%)	-	-	-	-	-
Hypomagnesemia	1 (2.9%)	-	-	-	-	-	-	-
Hyponatremia	1 (2.9%)	-	1 (2.9%)	-	-	-	1 (2.8%)	-
Hypophosphatemia	-	2 (5.7%)	-	-	-	-	-	-
Musculoskeletal and connective tissue disorders								
Arthralgia	3 (8.6%)	1 (2.9%)	1 (2.9%)	-	-	1 (2.8%)	1 (2.8%)	-
Back pain	-	-	-	-	-	-	1 (2.8%)	-
Bone pain	-	1 (2.9%)	-	-	-	-	-	-
Chest wall pain	-	-	-	-	1 (2.8%)	-	-	-
Muscle weakness lower limb	-	1 (2.9%)	-	-	-	-	-	-
Myalgia	4 (11.4%)	1 (2.9%)	-	-	-	-	-	-
Myositis	-	-	-	-	-	1 (2.8%)	-	-
Other : Arm Pain	1 (2.9%)	-	-	-	-	-	-	-
Other : Cramps	1 (2.9%)	-	-	-	-	-	-	-
Other : Hip Pain	-	1 (2.9%)	-	-	-	-	-	-

	Cohort 1				Cohort 2			
Organ class and AE names (CTCAE V4.03)	**Grade 1, N (%)**	**Grade 2, N (%)**	**Grade 3, N (%)**	**Grade 4, N (%)**	**Grade 1, N (%)**	**Grade 2, N (%)**	**Grade 3, N (%)**	**Grade 4, N (%)**
Other : Leg Pain	1 (2.9%)	-	-	-	-	-	-	-
Pain in extremity	-	-	-	-	-	1 (2.8%)	-	-
Nervous system disorders								
Dizziness	2 (5.7%)	-	-	-	-	-	-	-
Dysgeusia	1 (2.9%)	1 (2.9%)	-	-	1 (2.8%)	-	-	-
Headache	2 (5.7%)	3 (8.6%)	-	-	-	-	-	-
Lethargy	3 (8.6%)	2 (5.7%)	-	-	2 (5.6%)	2 (5.6%)	-	-
Presyncope	-	-	-	-	-	1 (2.8%)	-	-
Tremor	1 (2.9%)	-	-	-	-	-	-	-
Psychiatric disorders								
Insomnia	-	2 (5.7%)	-	-	-	-	-	-
Renal and urinary disorders								
Acute kidney injury	2 (5.7%)	2 (5.7%)	-	-	-	-	-	-
Other: Dysuria	-	-	-	-	1 (2.8%)	-	-	-
Other: Nephritis	-	-	-	-	-	-	1 (2.8%)	-
Proteinuria	-	-	-	-	1 (2.8%)	-	-	-
Reproductive system and breast disorders								
Pelvic pain	-	-	-	-	-	1 (2.8%)	-	-
Respiratory, thoracic and mediastinal disorders								
Cough	3 (8.6%)	-	-	-	-	-	-	-
Dyspnea	3 (8.6%)	4 (11.4%)	-	-	1 (2.8%)	1 (2.8%)	1 (2.8%)	-
Epistaxis	-	1 (2.9%)	-	-	-	-	-	-
Hoarseness	2 (5.7%)	-	-	-	-	-	-	-
Pneumonitis	1 (2.9%)	4 (11.4%)	2 (5.7%)	-	3 (8.3%)	-	-	-
Productive cough	2 (5.7%)	-	-	-	-	-	-	-
Voice alteration	-	-	-	-	1 (2.8%)	-	-	-
Skin and subcutaneous tissue disorders								
Dry skin	2 (5.7%)	-	-	-	2 (5.6%)	-	-	-
Erythema	-	-	1 (2.9%)	-	-	-	-	-
Hyperhidrosis	1 (2.9%)	-	-	-	1 (2.8%)	1 (2.8%)	-	-
Other: Rash Unspecified	6 (17.1%)	3 (8.6%)	2 (5.7%)	-	2 (5.6%)	-	-	-
Photosensitivity	-	-	-	-	1 (2.8%)	-	-	-
Pruritus	8 (22.9%)	1 (2.9%)	-	-	6 (16.7%)	-	-	-
Rash maculo-papular	6 (17.1%)	3 (8.6%)	3 (8.6%)	-	2 (5.6%)	-	-	-
Skin hypopigmentation	1 (2.9%)	-	-	-	-	-	-	-
Urticaria	1 (2.9%)	1 (2.9%)	-	-	-	-	-	-
Vascular disorders								
Hot flashes	-	1 (2.9%)	-	-	-	-	-	-
Hypertension	-	1 (2.9%)	-	-	-	-	-	-
Hypotension	-	2 (5.7%)	-	-	-	-	-	-
Vasculitis	-	-	1 (2.9%)	-	-	-	-	-

Table S4: Serious adverse events recorded within the two trial cohorts

	Cohort 1				Cohort 2			
Organ class and AE names (CTCAE V4.03)	**Grade 1 , N (%)**	**Grade 2, N (%)**	**Grade 3, N (%)**	**Grade 4, N (%)**	**Grade 1, N (%)**	**Grade 2, N (%)**	**Grade 3, N (%)**	**Grade 4, N (%)**
Endocrine disorders								
Adrenal insufficiency	-	-	-	1 (2.8%)	1 (2.9%)	-	-	-
Hyperthyroidism	1 (2.9%)	-	-	-	-	-	-	-
Hypothyroidism	-	-	-	1 (2.8%)	-	-	-	-
Eye disorders								
Retinal Vascular Disorder	-	-	-	-	1 (2.9%)	-	-	-
Uveitis	-	-	-	-	-	-	1 (2.9%)	-
Gastrointestinal disorders								
Colitis	-	1 (2.8%)	2 (5.7%)	-	5 (14.3%)	1 (2.8%)	-	-
Diarrhea	-	-	4 (11.4%)	1 (2.8%)	6 (17.1%)	1 (2.8%)	-	-
Nausea	-	-	1 (2.9%)	1 (2.8%)	-	-	-	-
Vomiting	-	-	2 (5.7%)	-	1 (2.9%)	1 (2.8%)	-	-
General disorders and administration site conditions								
Fatigue	-	-	1 (2.9%)	-	1 (2.9%)	-	-	-
Fever	2 (5.7%)	1 (2.8%)	1 (2.9%)	-	-	-	-	-
Immune system disorders								
Other: Hepatitis	-	-	1 (2.9%)	-	-	-	-	-
Other: Hypophysitis	-	-	-	-	1 (2.9%)	-	-	-
Other: Myastenia Gravis	-	-	-	-	-	1 (2.8%)	-	-
Other: Thyroiditis	-	-	1 (2.9%)	1 (2.8%)	-	-	-	-
Infections and infestations								
Anorectal Infection	-	-	-	-	1 (2.9%)	-	-	-
Investigations								
Alanine aminotransferase increased	-	-	-	-	-	1 (2.8%)	-	-
Aspartate aminotransferase increased	-	-	-	-	-	1 (2.8%)	-	-
Metabolism and nutrition disorders								
Anorexia	-	-	-	-	-	1 (2.8%)	-	-
Dehydration	-	-	1 (2.9%)	-	-	-	-	-
Hyperglycaemia	-	-	-	-	-	-	-	1 (2.8%)
Musculoskeletal and connective tissue disorders								
Arthralgia	-	-	-	-	1 (2.9%)	1 (2.8%)	-	-
Myositis	-	-	-	1 (2.8%)	-	-	-	-
Renal and urinary disorders								
Acute kidney injury	-	-	2 (5.7%)	-	-	-	-	-
Other: Nephritis	-	-	-	-	-	1 (2.8%)	-	-
Respiratory, thoracic and mediastinal disorders								
Dyspnoea	-	-	2 (5.7%)	-	-	1 (2.8%)	-	-
Pneumonitis	-	-	2 (5.7%)	-	2 (5.7%)	-	-	-
Skin and subcutaneous tissue disorders								
Other: Rash Unspecified	-	-	-	-	1 (2.9%)	-	-	-
Rash maculo-papular	-	-	-	-	1 (2.9%)	-	-	-
Vascular disorders								
Hypotension	-	-	1 (2.9%)	-	-	-	-	-
Vasculitis	-	-	-	-	1 (2.9%)	-	-	-

Supplementary Methods

Pre-screening tissue analysis.

Two formalin fixed paraffin embedded tumour tissue blocks were requested for testing of the immunogenic signatures. The block with the highest tumour content was selected for downstream processing. A minimum tumour content of 30% was required for achieving confident detection of genomic alterations and expression of mismatch repair proteins, and for excluding specimens that had high content of T Cells, but not actually infiltrating the tumour mass. Twenty slides between 3-5µm were prepared from the selected block.

Of the 71 ImS+ patients who received treatment within the trial, 10 had a fresh biopsy taken for the purpose of the trial, an on-treatment archival specimen was analysed for 7 additional patients, while archival diagnostic specimen, before initiation of systemic treatment, was analysed for the remaining 54.

Before DNA extraction for next generation sequencing was performed, all patients had histology reviewed for tissue qualification by EQK. TM quantified inflammatory infiltrate blinded to patient characteristics. MMR status report was issued by AF or AH. UW-Oncoplex reports were issued by CCP.

2.0 Supplementary results

Relevant treatments received before trial enrolment

Three patients in C2 received prior treatment with poly (ADP-ribose) polymerase (PARP) inhibitors, specifically one patient with ATM biallelic loss, one patient with MMRD and one patient with HII. No patients received prior platinum-based chemotherapy

Treatment discontinuation

Overall, 41/71 (58%) patients discontinued ipilimumab before completion of all 4 combination treatment cycles (22 and 19 in C1 and C2, respectively). Reasons for discontinuation were: i. adverse event (C1, n=20; C2, n=10); ii. disease progression (C1, n=2; C2, n=7); iii. patient's choice (C2 n=2). Of the 30 patients who discontinued ipilimumab due to toxicity, 10 (33%) had a response. The CRR among patients who completed all 4 cycles of combination ipilimumab and nivolumab was 13/32 (41%).