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Platinum Priority – Urothelial Cancer

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Phase 2 Trial of Gemcitabine, Cisplatin, plus Ipilimumab in Patients with Metastatic Urothelial Cancer and Impact of DNA Damage Response Gene Mutations on Outcomes

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Abstract

Background: Chemotherapy may exert immunomodulatory effects, thereby combining favorably with the immune checkpoint blockade. The pharmacodynamic effects of such combinations, and potential predictive biomarkers, remain unexplored.
Objective: To determine the safety, efficacy, and immunomodulatory effects of gemcitabine and cisplatin (GC) plus ipilimumab and explore the impact of somatic *DNA damage response* gene alterations on antitumor activity.
Design, setting, and participants: Multicenter single arm phase 2 study enrolling 36 chemotherapy-naïve patients with metastatic urothelial cancer. Peripheral blood flow cytometry was performed serially on all patients and whole exome sequencing of archival tumor tissue was performed on 28/36 patients.
Intervention: Two cycles of GC followed by four cycles of GC plus ipilimumab.
Outcome measurements and statistical analysis: *The* primary endpoint was 1-yr overall survival (OS). Secondary endpoints included safety, objective response rate, and progression-free survival.
Results and limitations: Grade $\geq$3 adverse events occurred in 81% of patients, the majority of which were hematologic. The objective response rate was 69% and 1-yr OS was 61% (lower bound 90% confidence interval: 51%). On exploratory analysis, there

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were no significant changes in the composition and frequency of circulating immune cells after GC alone. However, there was a significant expansion of circulating CD4 cells with the addition of ipilimumab which correlated with improved survival. The response rate was significantly higher in patients with deleterious somatic DNA damage response mutations (sensitivity = 47.6%, specificity = 100%, positive predictive value = 100%, and negative predictive value = 38.9%). Limitations are related to the sample size and single-arm design. ***Conclusions:*** GC + ipilimumab did not achieve the primary endpoint of a lower bound of the 90% confidence interval for 1-yr OS of >60%. However, within the context of a small single-arm trial, the results may inform current approaches combining chemotherapy plus immunotherapy from the standpoint of feasibility, appropriate cytotoxic *backbones*, and potential predictive biomarkers. Trial registration: ClinicalTrials.gov NCT01524991. ***Patient summary:*** Combining chemotherapy and immune checkpoint blockade in patients with metastatic urothelial cancer is feasible. Further studies are needed to refine optimal combinations and evaluate tests that might identify patients most likely to benefit.

1. Introduction

Metastatic urothelial cancer (UC) is a relatively chemotherapy-sensitive neoplasm with objective responses achieved in 50–60% of patients treated with cisplatin-based chemotherapy [1]. However, response durations are generally short and median survival is only ~14 mo [1]. Attempts to improve outcomes with additional cytotoxic agents have proven unsuccessful suggesting a therapeutic ceiling has been reached and highlighting the need for novel approaches [2].

Ipilimumab is a fully human monoclonal antibody directed against the immune checkpoint molecule cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) [3]. In a syngeneic murine bladder cancer model, CTLA-4 blockade induced tumor regression, improved survival, and increased levels of tumor-reactive T-cells [4]. A "window of opportunity" study demonstrated that ipilimumab induced immunomodulatory effects when administered prior to cystectomy in 12 patients with localized invasive UC [5]; however, the role of CTLA-4 blockade in metastatic UC has been underexplored.

Studies in model systems, and in patients, have demonstrated that cytotoxic chemotherapy may also exert immunomodulatory effects and therefore combine favorably with immune checkpoint blockade [6]. While the effects on the immune system are pleiotropic, chemotherapy can potentially augment tumor immunity via two key mechanisms: (1) by inducing immunogenic cell death (ie, the concomitant release of tumor antigens and danger associated molecular patterns such as high mobility group box 1 protein (HMGB-1), and/or (2) by direct modulation of the quantity and/or activity of immunosuppressive cellular subsets [6–10]. In syngeneic murine tumor models, combining ipilimumab with cytotoxic chemotherapy demonstrated synergistic antitumor activity accompanied by an increase in activated T-cells and a decrease in myeloid-derived suppressor cells [11].

Apart from the direct immunomodulatory effects of some cytotoxic agents, combining chemotherapy with immune checkpoint blockade could also represent an attractive strategy for patients with tumors harboring genomic alterations conferring sensitivity to both classes of therapies. The presence of somatic mutations in *DNA damage response* (*DDR*) genes has been correlated with response to cisplatin-based chemotherapy in UC [12–15]. Studies across various tumor types have demonstrated a correlation between higher tumor mutational load and response to immune checkpoint blockade [16,17]. Deleterious mutations in *DDR* genes may lead to hyper-accumulation of somatic mutations [18–20]. Therefore, tumors harboring somatic *DDR* mutations may be particularly vulnerable to the combination of cisplatin-based chemotherapy plus immune checkpoint blockade (Supplementary Fig. 1).

To better understand the potential role of combining chemotherapy plus immune checkpoint blockade, we designed a clinical-translational phase 2 study.

2. Patients and methods

2.1. Study design and treatment

Hoosier Cancer Research Network GU-148 was an investigator-initiated multi-center phase 2 trial. Both based on the hypothesis that chemotherapy administered first might induce immunogenic cell death, and to facilitate pharmacodynamic assessments, a *phased* schedule was employed (Supplementary Fig. 2). Patients received two cycles of gemcitabine (1000 mg/m^2 on days 1 and 8) plus cisplatin (70 mg/m^2 on day 1) every 21 d (GC). Patients subsequently received four cycles of GC plus ipilimumab (10 mg/kg on day 1) every 21 d. After completion of cycle 6, patients with at least stable disease could continue maintenance ipilimumab every 3 mo.

The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the local ethics committees at each participating site and informed consent was provided by all patients before enrollment.

2.2. Patients

Eligible patients were aged ≥18 yr and had metastatic UC of the bladder, urethra, ureters, or renal pelvis. Patients had received no prior systemic chemotherapy for metastatic disease; prior neoadjuvant/adjuvant therapy was permitted if completed ≥12 mo prior to registration. Patients were required to have adequate organ function and a Karnofsky performance status of at least 80%.

2.3. Study assessments

Tumor assessments were conducted using cross-sectional imaging of the chest, abdomen, and pelvis at screening, after cycle 2, after cycle 6, and every 3 mo. Response and progression were investigator assessed and were determined both by Response Evaluation Criteria in Solid Tumors

1.1 and by immune-related response criteria [21,22]. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0).

2.4. Immune monitoring

Methods for measuring peripheral blood immune subsets and HMGB-1 are outlined in the Supplementary data and Supplementary Table 1.

2.5. Whole-exome sequencing, mutational load, and DDR mutation analysis

Formalin-fixed paraffin-embedded archival tumor tissue was available for 28 of 36 patients enrolled on study (Supplementary Tables 2 and 3) and whole-exome sequencing (WES) was performed on the Illumina HiSeq 2500 or 4000 (Illumina, San Diego, CA, USA). The approach was as previously described [23] and described in detail in the Supplementary data.

2.5.1. DDR alterations and mutational load

Somatic *DDR* mutations were defined as protein-altering single nucleotide variants or indel somatic calls in 55 *DDR* genes (Supplementary Table 4) that were used in prior studies [12,18] or curated by the authors of this paper. Predicted deleterious mutations were defined as nonsense, frameshift, or affecting canonical splice site. Additionally, some missense mutations also were considered predicted deleterious based on manual review of evidence suggesting loss of function (summarized in Supplementary Table 5), incorporating the following criteria: location in a recurrently mutated *hotspot* in COSMIC v80 [24] or cBioPortal [25]; annotation in genetic disease in ClinVar [26] (accessed via https://www.ncbi.nlm.nih.gov/clinvar/ in April 2017); location in protein domain essential to its function. Missense mutations not judged to be deleterious were assigned to the *undetermined impact* category, meaning their functional impact is not known. Mutational load was defined as the number of protein-altering somatic single nucleotide variants calls identified by WES in hybridization capture target regions only (ie, genomic intervals not including the 100 nt padding previously described) [23]. Because indel variant calling, as well as calling of any variants in padding regions, have a higher false discovery rate, this strategy provided a more robust estimate of true mutational load. Additional details are provided in the Supplementary data.

2.6. Statistical analysis

The primary endpoint was 1-yr overall survival (OS) rate from initiation of GC. The secondary endpoints were safety, objective response rate, and progression-free survival (PFS). The null 1-yr OS rate was chosen to be 60% based on historical data from a large, phase 3 study randomizing patients to GC versus GC plus paclitaxel; this study enrolled 626 patients between May 2001 and June 2004 and the 1-yr OS rate was 52% and 61%, respectively [2]. The sample size was calculated for a power of 0.80, based on 90% one-sided confidence intervals (CI) calculated at a target rate of 80% for 1-yr OS. The combination regimen was to be recommended for further testing if the lower bound of the 90% CI exceeded 60%. This design (type I error level 0.10) required a sample size of 33 patients which was inflated to 36 patients to account for potential missing data. The sample size was determined from an upper bound for the Greenwood formula for the variance of Kaplan-Meier estimate at 1 yr [27].

3. Results

3.1. Patients and treatment

Between May 2012 and January 2015, 36 patients were enrolled at six centers. Baseline characteristics are shown in Table 1; 58% had visceral metastases and 47% had previously undergone definitive resection of their primary tumors. Patients received a median of six cycles of GC (range, 3–6) and four cycles of ipilimumab (range, 1–13). The most common reason for treatment discontinuation was disease progression. Ipilimumab maintenance was initiated by 8/36 (22%) patients.

Table 1 – Baseline characteristics

Characteristic	*N* = 36
Age, median (interquartile range)	64 (15)
Men, *n* (%)	29 (81)
Primary tumor, *n* (%)	
Bladder	28 (78)
Renal pelvis	7 (19)
Ureter	1 (3)
Karnofsky performance status, *n* (%)	
100%	9 (25)
90%	16 (44)
80%	11 (31)
Sites of metastatic disease, *n* (%)	
Lymph node/soft tissue	15 (42)
Visceral	21 (58)
Liver	7 (19)
Bajorin prognostic factors, *n* (%)	
0	9 (25)
1	22 (61)
2	5 (14)
Prior treatment, *n* (%)	
Systemic chemotherapy	5 (14)
Cystectomy or nephroureterectomy	17 (47)

3.2. Safety

Treatment-emergent adverse events occurring in ≥5% of patients are shown in Table 2; grade ≥3 adverse events occurred in 81% of patients and grade ≥3 adverse events, felt to be at least possibly related to treatment, occurred in 75% of patients. The majority of grade ≥3 adverse events were hematologic. The most common grade ≥3 immune-related adverse event was diarrhea occurring in 11% of patients. There were no treatment-related deaths.

3.3. Response rate and survival

The tumor response data are detailed in Table 3. Based on the *best responses* occurring at any time while on study, the objective response rate was 69% with 17% of patients achieving a complete response. A spider plot and swimmers plot demonstrating response kinetics and durations are shown in Figure 1A and Supplementary Figure 3, respectively. Unusual response kinetics were observed including late evolution of complete responses (Supplementary Fig. 4) and an outlier response in a patient with a *DDR* mutated tumor (Supplementary Fig. 5).

The 1-yr OS was 0.61 (lower bound 90% CI: 0.51; Fig. 1B). The median OS was 13.9 mo (95% CI: 10.5, 23.4). At 24 mo from initiation of treatment, 31% of patients (95% CI: 20%, 41%) were alive. The median PFS was 7.9 mo (95% CI: 6.4, 9.9).

Table 2 – Treatment-emergent adverse events occurring in ≥5% of patients (maximum grade per event per patient shown)

Adverse event	Grade 1, *n* (%)	Grade 2, *n* (%)	Grade 3, *n* (%)	Grade 4, *n* (%)
Any adverse event	1 (3)	6 (17)	19 (53)	10 (28)
Nonimmune related				
ALT increase	5 (14)	0	0	0
Alkaline phosphatase increased	4 (11)	1 (3)	0	0
Alopecia	10 (28)	0	0	0
Anemia	4 (11)	10 (28)	9 (25)	1 (3)
Anorexia	9 (25)	10 (28)	0	0
Arthralgia	4 (11)	0	0	0
Aspartate aminotransferase increased	4 (11)	0	0	0
Blurred vision	4 (11)	0	0	0
Chills	6 (17)	1 (3)	0	0
Constipation	17 (47)	7 (19)	0	0
Cough	16 (44)	2 (6)	0	0
Creatinine increased	7 (19)	3 (8)	1 (3)	1 (3)
Dehydration	2 (6)	3 (8)	2 (6)	0
Delirium	1 (3)	0	1 (3)	0
Depression	4 (11)	2 (6)	0	0
Dizziness	10 (28)	1 (3)	0	0
Dysguesia	8 (22)	3 (8)	0	0
Edema	8 (22)	3 (8)	0	0
Fatigue	16 (44)	14 (39)	3 (8)	0
Headache	8 (22)	4 (11)	0	0
Hyperglycemia	2 (6)	1 (3)	3 (8)	0
Hyperkalemia	2 (6)	2 (6)	0	0
Hypocalcemia	3 (8)	0	0	0
Hypokalemia	1 (3)	4 (11)	3 (8)	1 (3)
Hypomagnesemia	3 (8)	3 (8)	3 (8)	0
Hypophosphatemia	0	1 (3)	2 (6)	0
Lymphocyte count decreased	4 (11)	1 (3)	0	0
Mucositis	2 (6)	2 (6)	0	0
Nausea	13 (36)	13 (36)	1 (3)	0
Neutrophil count decreased	1 (3)	7 (19)	9 (25)	4 (11)
Peripheral sensory neuropathy	12 (33)	3 (8)	1 (3)	0
Platelet count decreased	8 (22)	2 (6)	4 (11)	3 (8)
Thromboembolic event	0	6 (17)	4 (11)	0
Tinnitus	3 (8)	1 (3)	0	0
Vomiting	9 (25)	4 (11)	1 (3)	0
White blood cell decreased	4 (11)	1 (3)	2 (6)	0
Immune related[a]				
Adrenal insufficiency	0	1 (3)	1 (3)	0
Colitis	1 (3)	1 (3)	3 (8)	0
Diarrhea	14 (40)	5 (14)	4 (11)	0
Hypophysitis	0	3 (8)	1 (3)	0
Hyperthyroidism	1 (3)	1 (3)	1 (3)	0
Hypothyroidism	1 (3)	3 (8)	0	0
Peripheral motor neuropathy	0	0	2 (6)	0
Pneumonitis	0	2 (6)	0	0
Pruritis	6 (17)	1 (3)	0	0
Rash aceneiform	3 (8)	3 (8)	1 (3)	0
Rash maculo-papular	5 (14)	1 (3)	1 (3)	0

ALT = alanine aminotransferase.
[a] Includes events that are possibly immune related.

3.4. *Immune monitoring*

No significant increase in serum HMGB1 levels were observed after treatment with two cycles of GC (Supplementary Figs. 6 and 7). The impact of GC alone, and GC plus ipilimumab, on circulating immune cells is shown in Table 4. There were no significant changes in immune cell subsets after GC alone including no significant depletion of CD4+ or CD8+ cells. After the addition of ipilimumab, there was a significant expansion of peripheral blood CD4+ and a numerical increase in peripheral blood CD8+ cells. No depletion of regulatory T cells or myeloid derived suppressor cells (MDSCs) was observed. An exploratory landmark analysis revealed a significant improvement in survival associated with a post-ipilimumab expansion of peripheral blood CD4+ cells (Fig. 1C).

3.5. *Impact of DDR mutations on mutational load and response*

The recurrently mutated genes from the gene list in Cancer Gene Census [24] are shown in Supplementary Figure 8; 29 somatic *DDR* mutations (among the 55 *DDR* genes defined in Supplementary Table 4) were identified (Supplementary Table 5). Sixteen of the 28 patients (57%)

Table 3 – Tumor response and disease control (*n* = 36)

	Response after cycle #2 (post-GC × 2 cycles alone), *n* (%)	Response after cycle #6 (post-GC + ipilimumab × 4 cycles), *n* (%)	Best response at any time while on study[a], *n* (%)
RECIST 1.1			
ORR	21 (58)	14 (39)	25 (69)
CR[a]	0	3 (8)	6 (17)
PR	21 (58)	11 (31)	19 (53)
SD	14 (39)	4 (11)	10 (28)
PD	1 (3)	4 (11)	1 (3)
Off treatment/Unknown	0	14 (39)	0
irRC			
irORR	23 (64)	15 (42)	27 (75)
irCR[a]	0	1 (3)	6 (17)
irPR	23 (64)	14 (39)	21 (58)
irSD	12 (33)	3 (8)	9 (25)
irPD	1 (3)	4 (11)	0
Off treatment/unknown	0	14 (39)	0

CR = complete responses; GC = gemcitabine plus cisplatin; ir = immune related; irRC = immune-related response criteria; ORR = objective response rate; PD = progressive disease; PR = partial responses; RECIST = Response Evaluation Criteria in Solid Tumors; SD = stable disease.

[a] Best responses at any time on study include responses recorded while patients on maintenance single-agent ipilimumab. Of note, 3/6 CRs occurred during follow-up after completing the concurrent chemotherapy + immune checkpoint blockade phase of the trial at ~9 mo, 17 mo, and 22 mo after initiation of cycle 1.

harbored at least one somatic *DDR* mutation and seven patients had multiple somatic *DDR* mutations. The mutational load was significantly higher in tumors harboring somatic *DDR* mutations than without (Fig. 2A).

Observation of the distribution of tumor mutation burden revealed a clearly separated bimodal distribution of protein-altering somatic mutation call counts (Fig. 2A). Among the four patients in the higher tumor mutation

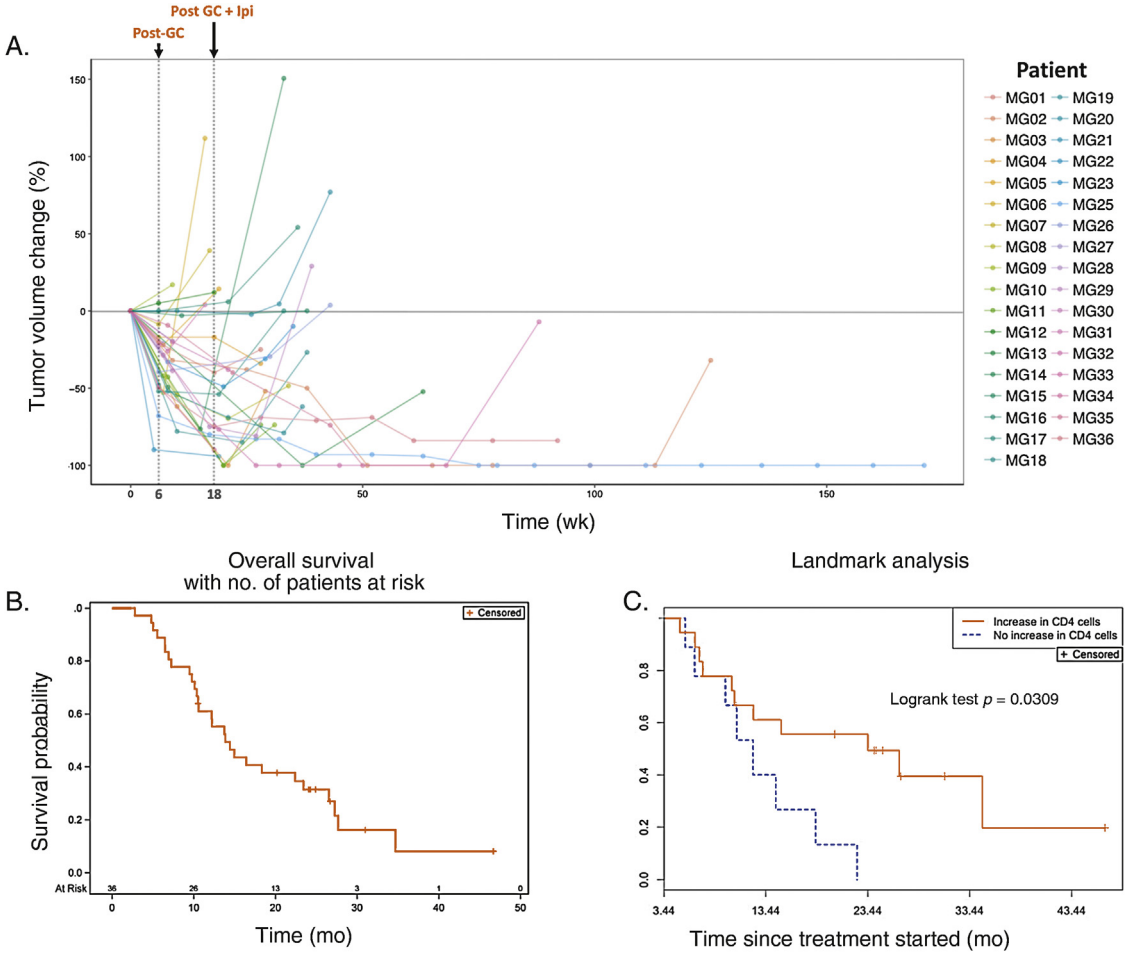

Fig. 1 – Survival outcomes in patients (*n* = 36) treated with gemcitabine, cisplatin (GC), plus ipilimumab (Ipi). (A) Spider plot demonstrating response kinetics, (B) overall survival, (C) landmark analysis for overall survival in patients with and without post-ipilimumab increases in peripheral blood CD4 + cells as measured by flow cytometry.

Table 4 – Impact of chemotherapy alone, and chemotherapy plus ipilimumab, on the composition and frequency of circulating immune cell subsets

Immune cell subset	Baseline (median, IQR) *n* = 36	Post GC[a] (median, IQR) *n* = 35	Post GC + Ipi[b] (median, IQR) *n* = 27	Wilcoxon signed-rank test *p* value		
				Post-GC versus baseline	Post-GC + Ipi versus post-GC	Post-GC + Ipi versus baseline
% CD4	8.6 (7.3)	9.7 (11.4)	11.4 (16.8)	0.1	0.047	0.004
Absolute CD4	643 (661)	749 (779)	947 (1339)	0.5	0.06	0.047
% CD8	4.0 (3.6)	4.2 (4.1)	5.8 (9.1)	0.8	0.2	0.1
Absolute CD8	304 (369)	297 (377)	370 (739)	0.7	0.4	0.4
% Tregs	0.4 (0.5)	0.6 (0.6)	0.7 (0.9)	0.06	0.043	0.006
% Gran MDSC	0.03 (0.09)	0.02 (0.08)	0.03 (0.06)	0.8	0.8	0.4
% Mono MDSC	0.01 (0.03)	0.01 (0.05)	0.02 (0.03)	0.6	0.3	0.9

GC = gemcitabine plus cisplatin; Gran MDSC = granulocytic myeloid derived suppressor cells; Ipi = ipilimumab; Mono MDSC = monocytic myeloid derived suppressor cells; Tregs = regulatory T cells.
% CD4, CD8, Tregs, and MDSCs per CD45 population.
[a] Post GC = post-2 cycles of gemcitabine plus cisplatin alone.
[b] Post-GC + Ipi = post three cycles of gemcitabine, cisplatin, plus ipilimumab.

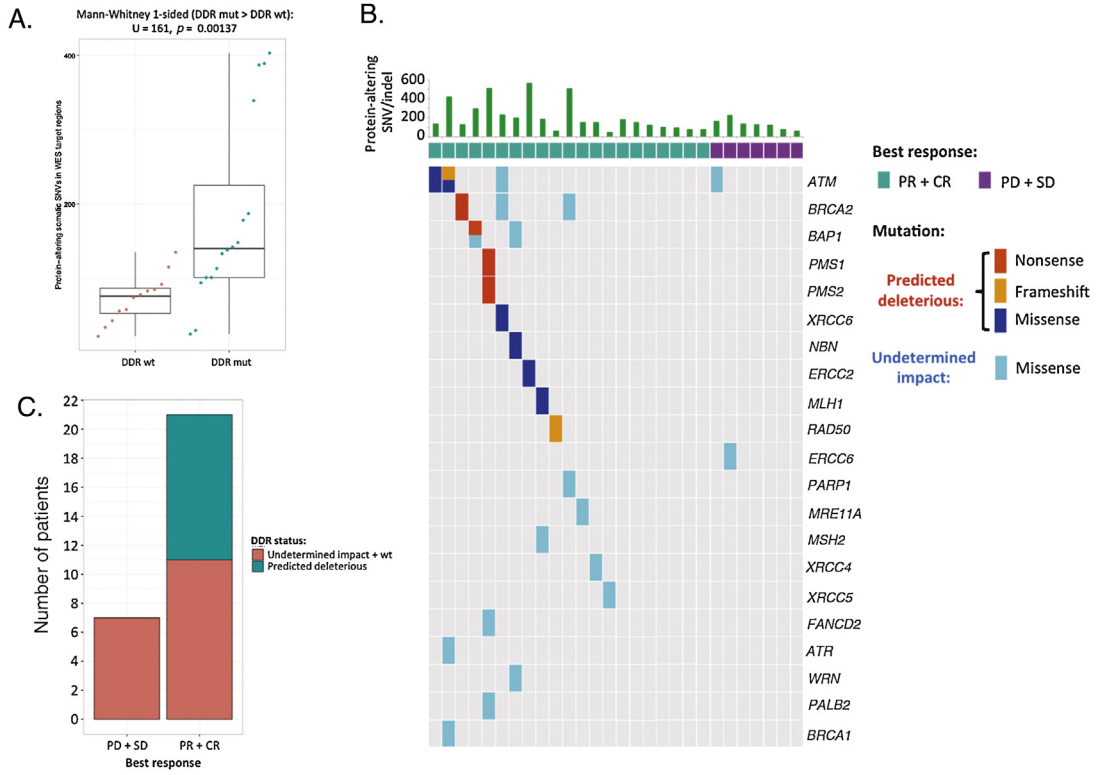

Fig. 2 – Association between *DNA damage response* gene (*DDR*) alterations as determined by whole exome sequencing and somatic mutational load or response to treatment (*n* = 28). (A) association between the presence of any protein-altering somatic *DDR* mutation and somatic single nucleotide variants (SNV) in whole exome sequencing target regions. (B) Oncoprint demonstrating landscape of somatic *DDR* mutations in cohort and (C) association between deleterious somatic *DDR* mutations and objective response to treatment.
CR = complete response; Mut = mutant; PD = progressive disease; PR = partial response; SD = stable disease; wt = wild type.

burden group, all achieved a partial or complete response to treatment (Supplementary Fig. 9).

Among the 16 patients with somatic *DDR* mutations, 10 were classified as having deleterious mutations (Fig. 2B, Supplementary Table 5). Utilizing the best response achieved while on study, patients with deleterious *DDR* mutations had a significantly higher response rate to treatment (two-sided Fisher's Exact Test $p = 0.03$) with all 10 patients with deleterious *DDR* mutations achieving a partial or complete response (Fig. 2B and C): sensitivity = 48% (95% CI: 26–70%), specificity = 100% (95% CI: 56–100%), positive predictive value = 100% (95% CI: 65.5–100%), and negative predictive value = 39% (95% CI: 18–64%). Patients with tumors harboring deleterious *DDR* mutations had numerically longer PFS and OS though this did not reach statistical significance (Supplementary Fig. 10).

4. Discussion

This was among the first trials initiated to explore immune checkpoint blockade in metastatic UC. However, during the conduct of this trial, the treatment landscape experienced a tectonic shift with proof of concept for immune checkpoint blockade with programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1) antibodies rapidly established [17,28,29]. This Renaissance era of immunotherapy in UC has fueled a rapidly expanding knowledge of immunobiology and a search for combination approaches to further improve outcomes including regimens integrating cytotoxic chemotherapy. Indeed, several randomized phase 3 trials have already been initiated exploring GC ± PD-1/PD-L1 blockade as first-line treatment for metastatic UC (NCT02807636, NCT02853305) without completed phase 2 studies. In this context, the current study provides several insights.

The administration of GC plus immune checkpoint blockade was feasible. Adverse events characteristic of both classes of drugs were observed but there was no evidence of *synergistic toxicity*; the majority of grade ≥3 adverse events were hematologic and consistent with the safety profile of GC. Immune-related adverse events were observed at frequencies similar to other studies utilizing ipilimumab. The study did not meet its primary endpoint for 1-yr OS of >60%. Though not a standard endpoint for a phase 2 trial, 1-yr OS rate was selected due to the phased treatment schedule employed as well as emerging data at the time indicating that CTLA-4 blockade may impact survival to a greater extent than intermediate endpoints. Indeed, unusual response kinetics and durable responses were observed. These findings, coupled with the observed pharmacodynamic changes, support further dissecting the role of ipilimumab in UC.

Combining chemotherapy with immune checkpoint blockade may be an attractive strategy for several reasons including: (A) direct modulation of immune cells, (B) induction of immunogenic cell death, and (C) shared genomic vulnerability to both classes of therapies. We sought to generate insights regarding these potential mechanisms in the clinic. While we did not observe significant depletion of MDSCs or regulatory T-cells in circulation with GC alone, we also did not observe significant depletion of CD4 and CD8 cells with GC, an initial concern with combining chemotherapy and immune checkpoint blockade. Indeed, with the addition of ipilimumab, we observed a significant expansion of peripheral blood CD4 cells that correlated with an improvement in survival on landmark analysis. Though changes in the peripheral blood may not recapitulate changes in the tumor microenvironment, serial tumor biopsies suffer from sampling bias and financial, ethical, and feasibility challenges. Further, our findings are highly aligned with recent data from organism-wide mass cytometry studies in murine models treated with immunotherapy demonstrating that an emergent population of peripheral CD4 cells is required for tumor eradication [30]. Though we detected very small numbers of MDSCs in circulation, there has been no universal definition of MDSC immunophenotype and we utilized a stringent definition likely accounting at least in part for this observation. Importantly, changes in peripheral blood immune subsets in this trial was an exploratory endpoint. The hypothesis, and immune cell panels, were specified a priori; however, due to the relatively small sample size and exploratory nature of the analysis, we did not conduct formal multiple test adjustments and the results need to be interpreted within this context and should be considered hypothesis generating.

In model systems, only certain cytotoxic agents have been shown to induce immunogenic cell death, a process characterized by calreticulin exposure, release of adenosine triphosphate, and release of HMGB-1 [31]. In the current study, treatment with GC alone did not result in a significant increase in serum HMGB-1. Importantly, studies across various experimental models demonstrate that cisplatin does not induce immunogenic cell death and further clinical-translational studies are likely required to optimize the chemotherapy *backbones* of combination regimens with immune checkpoint blockade [32].

To our knowledge, this is the first study to explore the concept that *DDR* mutations may render tumors particularly sensitive to *both* platinum-based chemotherapy and immune checkpoint blockade. Given that multiple genes are involved in *DDR*, and tools to predict the functional implications of specific somatic alterations have not been optimized or standardized, we pursued a very comprehensive approach employing WES and utilizing a predefined algorithm for functional predictions. Recently, Teo et al [33], using a targeted exome sequencing panel, reported that *DDR* alterations were associated with a significantly higher likelihood of response to single-agent PD-1/PD-L1 blockade in patients with metastatic UC. Together, these findings are ideally suited for validation in ongoing trials randomizing patients to immune checkpoint blockade versus GC versus GC plus immune checkpoint blockade in an effort to identify a subset of patients that might particularly benefit from the combination approach.

There are limitations to our study particularly related to the small size, single-arm design, inclusion of limited enrollment sites, and potential patient heterogeneity. However, at the time that the study was designed, immune checkpoint blockade was *off the radar* in metastatic UC and our goal was to detect a signal of activity and begin to probe the mechanistic basis for combination therapy. Archival tumor tissue used for WES was derived from primary tumors in the vast majority of patients but represented a mix of biopsy specimens and specimens from definitive surgeries (eg, cystectomy). Our translational studies are hypothesis generating but provide several leads for follow-up in ongoing large randomized trials. Finally, we explored CTLA-4 blockade at a time when PD-1/PD-L1 antibodies were still in early phase clinical development. PD-1/PD-L1 blockade has emerged a key therapeutic class for metastatic UC. Still, the success of PD-1/PD-L1 blockade has led to a renewed interest in understanding the role of CTLA-4 blockade alone, and in combination with PD-1/PD-L1 blockade, in UC. Recently, an objective response rate of 18.8% with single agent CTLA-4 blockade was reported in

patients with metastatic UC further supporting the potential contribution of CTLA-4 blockade to the unusual response kinetics and favorable outcomes of a subset of patients treated on the current study [34].

5. Conclusions

Cytotoxic chemotherapy and immune checkpoint blockade, when administered independently, are now the two pillars of systemic therapy for treatment of advanced UC based on prospective trials, regulatory approvals, and treatment guidelines. The current trial may provide insights regarding refining the use of these therapeutic classes when given in combination. Presented in part as an oral presentation at the American Society of Clinical Oncology Genitourinary Cancers Symposium 2016.

Author contributions: Matthew D. Galsky had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.eururo.2017.12.001.

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