Original Study

Clinical Evaluation of Cisplatin Sensitivity of Germline Polymorphisms in Neoadjuvant Chemotherapy for Urothelial Cancer

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Abstract

To identify patients with urothelial cancer most likely to benefit from neoadjuvant chemotherapy, we evaluated germline pharmacogenomic markers for an association with response in 205 patients across 3 institutions. Stage pT0 (26%) and < pT2 (50%) rates were consistent across the respective discovery and replication cohorts. Despite the large effects for 3 polymorphisms in the discovery set, none were associated with achievement of pT0 or < pT2 on replication. Multi-institutional efforts are feasible and will be necessary to achieve advances in urothelial cancer precision medicine.

Background: Level 1 evidence has demonstrated increased overall survival with cisplatin-based neoadjuvant chemotherapy for patients with muscle-invasive urothelial cancer. Usage remains low, however, in part because neoadjuvant chemotherapy will not be effective for every patient. To identify the patients most likely to benefit, we evaluated germline pharmacogenomic markers for association with neoadjuvant chemotherapy sensitivity in 2 large cohorts of patients with urothelial cancer. **Patients and Methods:** Patients receiving neoadjuvant cisplatin-based chemotherapy for muscle-invasive urothelial cancer were eligible. Nine germline single nucleotide polymorphisms (SNPs) potentially conferring platinum sensitivity were tested for an association with a complete pathologic response to neoadjuvant chemotherapy (pT0) or elimination of muscle-invasive cancer (<pT2). **Results:** The data from 205 patients were analyzed—59 patients were included in the discovery set and 146 in an independent replication cohort—from 3 institutions. The stage pT0 (26%) and < pT2 (50%) rates were consistent across the discovery and replication populations. Using a multivariate recessive genetic model, rs244898 in *RARS* (odds ratio, 6.8; 95% confidence interval, 1.8-28.9; $P = .006$) and rs7937567 in *GALNTL4* (odds ratio, 4.8; 95% confidence interval, 1.1-22.6; $P = .04$) were associated with pT0 in the discovery set. Despite these large effects, neither were associated with achievement of pT0 in the replication set. A third SNP, rs10964552, was associated with stage < pT2 in the discovery set but also failed to replicate. **Conclusion:** Germline SNPs previously associated with platinum sensitivity were not associated with the neoadjuvant chemotherapy response in a large replication cohort of patients with urothelial cancer. These results emphasize the need for replication when evaluating pharmacogenomic markers and demonstrate that multi-institutional efforts are feasible and will be necessary to achieve advances in urothelial cancer pharmacogenomics.

Clinical Genitourinary Cancer, Vol. ■, No. ■, ■-■ © 2016 Elsevier Inc. All rights reserved.
Keywords: Bladder cancer, Pharmacogenomics, Precision medicine, Treatment response prediction

Introduction

Despite level 1 evidence demonstrating a survival benefit for cisplatin-based neoadjuvant chemotherapy in urothelial cancer,[1-3] its usage has historically been low.[4-6] Cisplatin-based neoadjuvant chemotherapy will not be effective for every patient—approximately one half will demonstrate disease downstaging to non—muscle-invasive disease, and approximately one third will achieve a complete pathologic response.[1,7-9] However, in those who do achieve a complete pathologic response (pT0), overall survival has been dramatically improved, independent of the initial clinical stage or other clinical factors, with 85% of those attaining pT0 alive at 5 years compared with 45% of those not achieving a complete response.[1] The likelihood of achieving pT0 is about 2.5 times greater with receipt of neoadjuvant chemotherapy.[1]

These data invite the proposition that the neoadjuvant setting could be an ideal clinical niche in which to investigate predictive chemotherapy-response biomarkers, with the goals of better patient selection to lead to an improved therapeutic index.[10] Patients unlikely to respond to cisplatin-based therapy could proceed directly to cystectomy or be considered for novel neoadjuvant treatments.

Our project sought to apply the rapidly evolving genomic knowledge to this question, with the hypothesis that germline genetic polymorphisms are potentially important predictors of the cisplatin response in urothelial cancer. Most previous studies of bladder cancer have focused on tumor genomics (ie, somatic mutations, such as *p53* and *ERCC1/2*) rather than germline genetic variation (inherited DNA polymorphisms) as determinants of the chemotherapy response. However, the importance of germline polymorphisms in governing drug levels and disposition, toxicity, and response has long been recognized in oncology (*TPMT* polymorphisms with 6-mercaptopurine and *UGT1A1* polymorphisms with irinotecan are salient examples).[11] In bladder cancer, we previously examined a large list of germline polymorphisms from candidate genes hypothesized to have effects on cisplatin sensitivity and tested these in a heterogeneous population of platinum-treated patients.[12] Although several single nucleotide polymorphisms (SNPs) correlated with the response, the findings were not replicated,[12] and the model did not focus on the uniquely relevant neoadjuvant setting.

Given the key role of cisplatin in the treatment of urothelial cancer, the question of a genetic predisposition to a response to cisplatin-based chemotherapy deserves attention as one of high clinical importance. In the present study, we sought to identify and replicate novel germline polymorphisms of interest in the cisplatin response in 2 large populations of patients with urothelial cancer receiving cisplatin-based neoadjuvant chemotherapy. The pathologic disease response in the surgical specimen was the primary endpoint.

Patients and Methods

Patients

The members of the institutions participating in this project (Fox Chase Cancer Center [FCCC], Memorial Sloan Kettering Cancer Center [MSKCC], and The University of Chicago [Chicago]) collected germline DNA samples and clinical follow-up data from patients with urothelial cancer treated with neoadjuvant chemotherapy. The respective institutional review boards approved the protocols, including a study funded and designed specifically for this purpose (ClinicalTrials.gov identifier, NCT01206426). To be included, patients must have had muscle-invasive urothelial carcinoma (stage ≥ cT2), received ≥ 3 cycles of chemotherapy in the neoadjuvant setting, consisting of a regimen with either GC (gemcitabine/cisplatin) or MVAC (methotrexate/vinblastine/doxorubicin/cisplatin), and undergone definitive surgery (ie, bladder, upper tract, and urethra primary permitted). Patients with pure variant histologic types were excluded (mixed histologic types were included as long as the predominant component was urothelial carcinoma). Patients with clinically apparent positive nodes before neoadjuvant chemotherapy were excluded. Germline DNA was isolated from peripheral blood (Chicago, FCCC) or saliva (MSKCC) samples. In assembling the discovery and replication cohorts, the enrolled patients with germline DNA that had already been extracted and ready for analysis were included in the first (discovery) cohort (all from MSKCC). The remaining patients were, by definition, included in the replication cohort, including patients from Chicago and FCCC and any MSKCC patients not included in the discovery cohort.

SNP Selection

Previous germline investigation of platinum sensitivity has centered primarily on candidate genes—genes hypothesized to modulate cisplatin sensitivity because of their putative role in the drug's mechanism of action. These efforts have largely focused on genes involved in DNA repair.[13,14] Such studies, including those of urothelial cancer, have been unable to consistently replicate any germline polymorphisms. We therefore intended to apply a different approach to the question. We used genome-wide methods to select the SNPs for testing—thus not confining the analysis to the supposition that important platinum sensitivity SNPs are located in "traditional" candidate genes.

We previously used and refined a novel cell-based genome-wide method to identify the germline genetic variants governing chemotherapy susceptibility[15] specifically for platinum drugs.[16,17] This in vitro model uses well-genotyped lymphoblastoid cell lines from healthy individuals in the International HapMap Project,[18] which were then treated with platinum to produce individual "sensitivity phenotypes." Next, genome-wide association studies were performed to associate platinum susceptibility with specific SNPs. The associated SNPs represent potentially novel genetic determinants of platinum sensitivity, identified from across the genome (unbiased approach) and often in genomic regions not previously implicated.

We selected 10 SNPs with the greatest quality associations from these previous studies for testing in the present study. Five of these (rs2191934, rs9527419, rs244903, rs7210837, rs3893319) were strongly associated in a large cell-based genome-wide meta-analysis of 608 human germline DNA samples treated with platinum compounds to determine the sensitivity.[17] All 5 were among the top statistical signals, with rs2191934 (meta $P = 8.3 \times 10^{-5}$) and rs9527419 (meta $P = 5.8 \times 10^{-6}$) specifically found to (distantly) regulate the expression of *GSTT1*, *ERCC6*, and *ERCC2*,

respectively. However, the SNPs themselves were not located in any of these genes,[17] potentially the reason they were missed by traditional candidate gene analyses. Separately, rs7937567 had been identified and replicated in a previous cell-based genome-wide study[16] and is located in an intron of *GALNTL4*, which was implicated twice in separate cell-based platinum-sensitivity studies.[16,19] The latter also implicated rs2136241 (*CDCA1* promoter SNP) and rs1649942 (intron of *NRG3*), both of which we selected. rs1649942 was also shown to be significantly associated with survival in a clinical cohort of patients with carboplatin-treated ovarian cancer.[20] rs10964552, located in *MLLT3*, was selected, because it was found as a top signal in a previous cell-based genome-wide study and was shown to regulate expression of *HIST1H3A*, a histone component, with greater HIST1H3A levels associated with platinum resistance.[16] Finally, rs6870861 was associated with platinum sensitivity in both a large cell-based genome-wide study and a cohort of patients with head and neck cancer,[21] and SNPs in linkage disequilibrium were shown to transregulate *SLC22A5*, a member of the organic cation transporter family intensively studied in platinum handling.[21]

Genotyping

Genotyping was performed using the MassARRAY iPLEX system (Sequenom, Inc.). For rs244903, a SNP in complete linkage disequilibrium in whites (rs244898) was genotyped as the proxy owing to design limitations with rs244903. One SNP (rs7210837) was unable to be successfully genotyped because of primer failure, leaving 9 SNPs tested in all patients. As an assessment of DNA quality, the call rates across the tested SNPs in the discovery and replication sets exceeded 97.7% and 98.8%, respectively.

Phenotype Definition and Association Analysis

We evaluated the association of the preselected germline pharmacogenomic markers with the neoadjuvant therapy outcomes. For each platinum susceptibility SNP of interest, the pathologic disease response at surgery was compared among the individuals according to the genotype at that SNP, allowing the identification of genotypes associated with cisplatin susceptibility/resistance. An association with a complete pathologic response (pT0 rate) at surgery was the primary endpoint. Downstaging after neoadjuvant chemotherapy (< pT2 rate) was the secondary endpoint. Surgical staging was assigned by dedicated pathologists for clinical purposes with no knowledge of the patients' genomic information. The personnel performing genotyping were also kept unaware of the surgical outcomes until after the genotypes had been assigned.

Statistical Analysis

In the discovery cohort, univariate and multivariate logistic regression analyses were conducted to investigate the association between each SNP and the pT0 and < pT2 rates. Recessive, dominant, and additive genetic models were tested. Because replication in a second, independent population was conducted, the *P* value for nominal significance in the discovery set was not adjusted for multiple testing correction and was chosen as *P* < .05, the threshold for a SNP to be considered promising and thus carried forward for testing in the replication set. Before replication

Table 1	Clinical Characteristics of the Discovery and Validation Populations	
Variable	**Discovery Cohort (n = 59)**	**Validation Cohort (n = 146)**
Age (years)		
Median	64	65
Range	31-86	32-83
Gender		
Male	40 (68)	104 (71)
Female	19 (32)	42 (29)
Institution		
MSKCC	59 (100)	92 (63)
FCCC	—	33 (23)
Chicago	—	21 (14)
Primary site		
Bladder	59 (100)	141 (97)
Upper tract	—	4 (3)
Synchronous	—	1 (<1)
Clinical stage		
≥cT2, N0	59 (100)	146 (100)
Treatment		
GC	55 (93)	89 (61)
GC plus sunitinib	3 (5)	7 (5)
MVAC	1 (2)	—
DD GC	—	7 (5)
GC → gemcitabine/carboplatin	—	1 (<1)
DD MVAC	—	42 (29)
Pathologic response		
pT0	15 (25)	38 (26)
<pT2	16 (27)	35 (24)
≥pT2	28 (48)	73 (50)

Data presented as n (%), unless noted otherwise.
To eliminate confounding by population stratification, only samples from self-identified whites were included in the primary analyses (discovery and replication).
Abbreviations: DD = dose dense; FCCC = Fox Chase Cancer Center; GC = gemcitabine/cisplatin; Chicago = University of Chicago; MVAC = methotrexate/vinblastine/Adriamycin/cisplatin; MSKCC = Memorial Sloan Kettering Cancer Center.

testing, formal sample size analysis was undertaken to determine the replication cohort size needed to have adequate power to replicate the SNPs. Using 10,000 simulated data sets, it was calculated that ≥ 134 patients in the replication cohort would provide 80% power to detect the effects of SNPs selected independently from the discovery cohort at *P* = .05, assuming odds ratios (ORs) and minor allele frequencies equivalent to those in the discovery set.

It was prespecified that in the validation set, even if the genetic relationship did not necessarily follow the same (eg, recessive) model, we would still examine for genetic effects using log-additive and dominant models. The power of these analyses was expected to be even greater.

In both the discovery and the replication sets, the secondary analysis was also prespecified to test the association between the response rate and the combination of SNPs (number of favorable

genotypes in each patient), using a trend test. In this analysis, the number of favorable genotypes carried by each patient was considered. In the simplest 2-SNP model (which was ultimately used), each patient was coded as carrying both, 1 and only 1, or neither favorable genotype, and the code was incorporated as $t = (2, 1, 0)$ in an additive model.[22]

To eliminate confounding by population stratification,[23] only samples from self-identified white patients were included in the primary analyses (discovery and replication sets). All SNPs were in Hardy-Weinberg equilibrium.

Results

Discovery Population

For the first analysis of the 9 preidentified SNPs, platinum sensitivity (defined by the pT0 and < pT2 rates) was tested in a single-institution discovery cohort of 59 patients. The clinical features of the discovery cohort are listed in Table 1. The pathologic complete response (pT0) rate in this cohort was 25.4%, and the disease of 52% of the patients was downstaged (< pT2) at surgery after the receipt of chemotherapy.

Using a recessive genetic model, rs244898 in *RARS* (OR, 6.8; 95% confidence interval [CI], 1.8-28.9; univariate $P = .006$) and rs7937567 in *GALNTL4* (OR, 4.8; 95% CI, 1.1-22.6; univariate $P = .04$) were associated with the likelihood of achieving pT0. For each SNP, 56% of the patients with the favorable genotype achieved pT0 (Figure 1, A and B). Demonstrating the apparent independent nature of the 2 SNPs, patients with either favorable genotype had pT0 with an OR of 8.5 (95% CI, 2.5-31.8; $P = .0008$). The combined effect of testing for both SNPs was also highly informative, because 2 of the 3 patients with both favorable genotypes achieved pT0 (67%) compared with 8 of 15 patients with 1 favorable genotype (53%), and only 5 of 41 achieving pT0 among those who lacked both favorable genotypes (12%; Figure 1C). The negative predictive value considering both SNPs in the discovery cohort was 88%.

For analysis of the secondary endpoint (< pT2), 1 SNP was significantly associated with downstaging at cystectomy (using an additive genetic model): rs10964552 (in *MLLT3*) with an OR of 5.0 (95% CI, 1.4-20.5; univariate $P = .02$). Expressed another way, the likelihood of no response to cisplatin-based neoadjuvant therapy

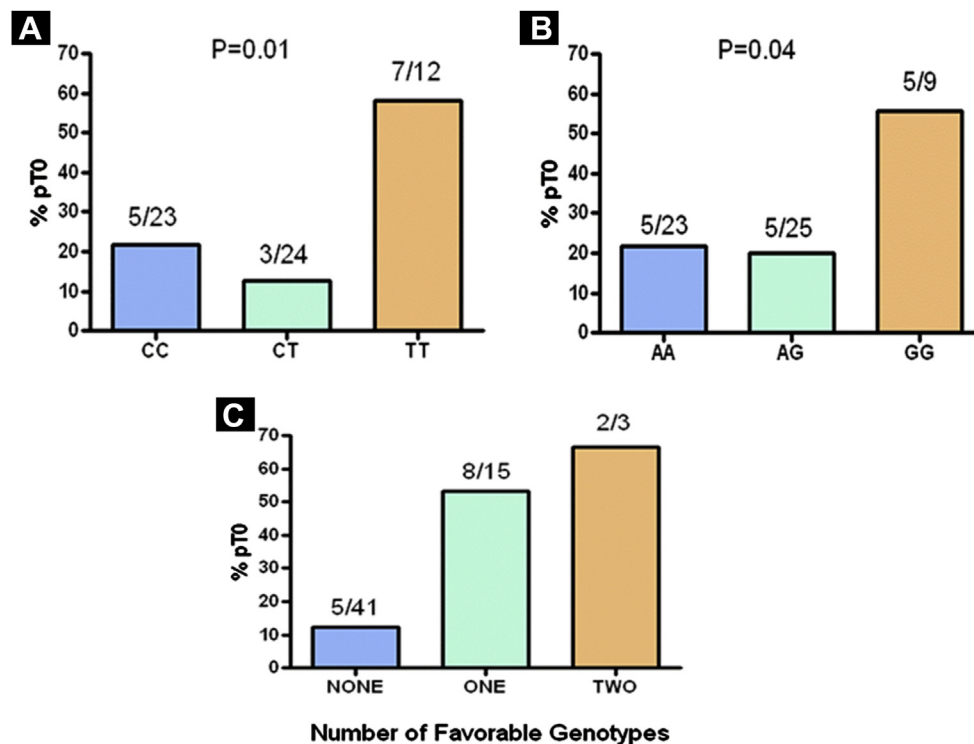| Figure 1	Positive Associations of Single Nucleotide Polymorphisms (SNPs) in the Discovery Population (n = 59). (A) Association of rs244898 With pT0 Rate, Using a Recessive Genetic Model. Patients Carrying Both Favorable Alleles (TT) Had a pT0 Rate of 58%. rs244898 Is an Intron SNP in *RARS*, With a Frequency in Whites of 0.44. rs244898 Is in Linkage Disequilibrium With an Exon Missense SNP for This Gene. (B) Association of rs7937567 With pT0 Rate, Using a Recessive Genetic Model. Patients Carrying Both Favorable Alleles (GG) Had a pT0 Rate of 56%. rs7937567 Is an Intron SNP in *GALNTL4*, With a Frequency in Whites of 0.42. (C) Two-SNP Model Incorporating Both rs244898 and rs7937567 Examining the Effect of 0, 1, or 2 Favorable Genotypes on the Pathologic Response Rate (pT0). Patients With Either the rs244898 TT Genotype or the rs7937567 GG Genotype Had a > 50% Rate of pT0. Patients Lacking Both Favorable Genotypes Were Highly Unlikely to Achieve pT0 (12%)

Figure 2 Positive Single Nucleotide Polymorphisms (SNP) Association With Downstaging at Cystectomy After Neoadjuvant Chemotherapy in the Discovery Population (n = 59). The Likelihood of No Response to Cisplatin-Based Neoadjuvant Therapy Was Significantly Greater in Patients Carrying the A Allele of rs10964552. rs10964552 Is an Intron SNP in *MLLT3*, With a Frequency in Whites of 0.17
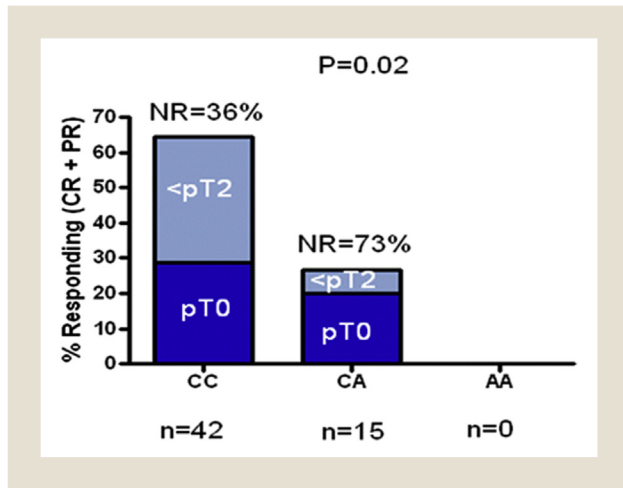
was significantly greater for patients carrying the A allele for rs10964552 (Figure 2).

The full SNP association results, including the direction of the clinical effect by allele for both the primary (pT0) and the secondary (< pT2) clinical endpoints, are listed in Table 2.

Replication Population

The 2 SNPs associated with pT0 and the third SNP associated with downstaging to < pT2 were then tested in the multi-institutional independent validation cohort of 146 patients (we were able to recruit even more patients than the 134 required by the minimum power calculation threshold for replication). The clinical features of the replication population are listed in Table 1. The rates

of pT0 and < pT2 were 26% and 50%, respectively, comparable to those of the discovery population.

For the analysis of the primary endpoint in the replication population, regression was performed on pT0 stratified by whether the patients carried the favorable genotypes—TT for rs244898 and GG for rs7937567. Although each SNP had an OR of effect of approximately 5 on pT0 in the discovery set, neither was associated with achievement of pT0 in the replication set (rs244898 replication cohort, OR, 1.1; $P = .79$; rs7937567 replication cohort, OR, 0.6; $P = .42$). The 2 SNPs combined (rs244898 and rs7937567 in 1 model) also were not significant. The third SNP (rs10964552), which was associated with pathologic downstaging to < pT2 in the discovery set, also failed to replicate (replication cohort, OR, 0.9; $P = .69$).

Given the possibility that differences in the treatment regimens might have confounded the replication (a much greater percentage of patients received dose-dense MVAC in the replication cohort than in nearly exclusively GC-treated discovery cohort), we performed a subanalysis of only the GC-treated validation cohort patients. Nonetheless, we did not find replication of any SNPs. The genotype frequencies were not significantly different between the discovery and replication cohorts: rs244898 discovery/replication, CC = 0.39/0.26, CT = 0.41/0.54, TT = 0.20/0.20; rs7937567, AA = 0.40/0.36, AG = 0.44/0.50, GG = 0.16/0.14; and rs10964552, CC = 0.74/0.72, CA = 0.26/0.26, AA = 0.00/0.02.

Discussion

Given that the chemotherapy survival benefit occurs in a few patients but that all patients are exposed to very substantial toxicities, the clinical benefit in this population of patients would be markedly enhanced if we could restrict chemotherapy to those patients most likely to benefit. The era of genomics offers a ripe avenue for this type of pursuit. Recent advances have begun to address this problem, identifying tumor-based genomic markers predictive of the cisplatin-based chemotherapy response in bladder cancer,[24-28] including an elegant investigation performed in the neoadjuvant setting.[29] It is likely that both somatic and germline factors govern chemotherapy responses.

Table 2	Summary of Genotype Association Results in the Discovery Cohort				
SNP	Recessive Allele (Frequency)[a]	Association With pT0[b]		Association With <pT2 (Downstaging)[c]	
		OR	*P* Value	OR	*P* Value
rs10964552	A (0.13)	NA	NA	0.20[d]	.017[d]
rs1649942	C (0.20)	0.00	.994	1.02	.963
rs2136241	C (0.46)	0.82	.795	1.08	.832
rs2191934	T (0.47)	0.42	.300	1.26	.535
rs244898	T (0.41)	6.82[d]	.006[d]	1.60	.192
rs3893319	G (0.05)	NA	NA	1.93	.471
rs6870861	C (0.11)	0.00	.992	0.38	.131
rs7937567	G (0.38)	4.75[d]	.040[d]	0.95	.885
rs9527419	T (0.14)	NA	NA	1.09	.871

Abbreviations: NA = not applicable for these results (ORs and *P* values were not provided because these were test instances in which either 0 or only 1 homozygous recessive patient was present in the cohort; ORs and *P* values for these situations in a recessive model were therefore undependable); OR = odds ratio; SNP = single nucleotide polymorphism.
[a]Allele frequency in the discovery cohort of the present study.
[b]Using recessive genetic model; ORs calculated using the major allele as the reference (comparator).
[c]Using additive genetic model; the OR is expressed for the impact of carriage of increasing copies of the minor allele.
[d]Statistically significant.

We investigated germline SNPs identified by unbiased genome-wide association study approaches. These SNPs all had published evidence of an association with platinum responsiveness, some with strong mechanistic plausibility.[16,17,19-21] Despite intriguing associations for 3 of these SNPs in the discovery population of the present study, none were replicated in our well-powered validation cohort.

Several reasons for this were considered. First, although the discovery and replication cohorts were similar in nearly all measured demographic variable, the discovery population was derived from a single institution, and the replication cohort was assembled from 3 centers. We viewed the latter as a strength, and replication across institutions would have increased the generalizability. However, the lack of replication might have reflected unmeasured clinical differences between the cohorts, including patient or practice differences. Also, a greater proportion of (dose-dense) MVAC use occurred in the validation set, and this could have hindered replication through a mechanism by which platinum-specific genetic effects can be obscured in a 4-drug regimen. However, post hoc analysis of only the GC-treated validation cohort did not find any replication. Replication failure could have simply demonstrated that the findings in the discovery cohort were spurious associations. This is certainly possible, given the small size of the discovery cohort. (Perhaps consistent with this idea was the finding that the discovery SNPs associated with pT0 were also not found to be associated with pathologic downstaging [< pT2 in the discovery set.) Although the SNPs tested were previously associated in other models, the relevance of those previous cell-based models to the clinical treatment response remains unproved.

The strengths of our study included the carefully defined and highly relevant clinical endpoint of a complete pathologic response, prospective identification of samples for inclusion, and execution of a formal power analysis to predetermine the required sample size for replication before replication testing (to decrease the likelihood that negative findings had resulted from an underpowered analysis).

Our goal was to improve overall survival and spare potential toxicity in individuals unlikely to benefit from the therapies. One method of accomplishing this will be the development of newer, perhaps better-tolerated, drugs. Simultaneously, we should continue to strive for a better understanding of the genetic factors governing platinum-based chemotherapy in urothelial cancer, because even the advent of new therapies is only likely to add to this traditional backbone of therapy for this challenging disease.

Conclusion

We investigated germline SNPs implicated as potentially governing platinum responsiveness but were unable to replicate the association of these SNPs with achievement of a pathologic response after neoadjuvant cisplatin-based chemotherapy for urothelial cancer in > 200 treated patients from 3 institutions. Our results emphasize the importance of replication when evaluating pharmacogenomic markers. We demonstrated that multi-institutional collaborations are feasible and necessary to achieve advances in urothelial cancer pharmacogenomics. Through this existing collaboration, we are now pursuing a follow-on genome-wide study to identify new germline polymorphisms of platinum chemotherapy response in urothelial carcinoma.

Clinical Practice Points

- Level 1 evidence has demonstrated increased overall survival with cisplatin-based neoadjuvant chemotherapy for patients with muscle-invasive urothelial cancer; however, usage has remained low, in part because neoadjuvant chemotherapy has not been effective for every patient.
- To improve the selection of patients most likely to benefit, we evaluated the germline pharmacogenomic markers for association with neoadjuvant chemotherapy sensitivity in 2 large cohorts of patients with urothelial cancer.
- Nine germline SNPs potentially conferring platinum sensitivity were tested for association with a complete pathologic response to neoadjuvant chemotherapy (pT0) or elimination of muscle-invasive cancer (< pT2).
- The data from 205 patients were analyzed—59 patients in the discovery set and 146 in an independent replication cohort—from 3 institutions.
- The pT0 (26%) and <pT2 (50%) rates were consistent across discovery and replication populations.
- Using a multivariate recessive genetic model, rs244898 in *RARS* (OR, 6.8; 95% CI, 1.8-28.9; *P* = .006) and rs7937567 in *GALNTL4* (OR, 4.8; 95% CI, 1.1-22.6; *P* = .04) were associated with pT0 in the discovery set.
- Despite these large effects, neither was associated with achievement of pT0 in the replication; a third SNP, rs10964552, was associated with < pT2 in the discovery set but also failed to replicate.
- These results emphasize the need for replication when evaluating pharmacogenomic markers and have demonstrated that multi-institutional efforts are feasible and will be necessary to achieve advances in urothelial cancer pharmacogenomics.

Disclosure

The authors have stated that they have no conflicts of interest.

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