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.

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## 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 $\geq$ cT2), received $\geq$ 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*,