Cumulative Genetic Risk Predicts Platinum/Taxane-Induced Neurotoxicity

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Abstract

Purpose: The combination of a platinum and taxane are standard of care for many cancers, but the utility is often limited due to debilitating neurotoxicity. We examined whether single-nucleotide polymorphisms (SNP) from annotated candidate genes will identify genetic risk for chemotherapy-induced neurotoxicity.

Patients and Methods: A candidate–gene association study was conducted to validate the relevance of 1,261 SNPs within 60 candidate genes in 404 ovarian cancer patients receiving platinum/taxane chemotherapy on the SCOTROC1 trial. Statistically significant variants were then assessed for replication in a separate 404 patient replication cohort from SCOTROC1.

Results: Significant associations with chemotherapy-induced neurotoxicity were identified and replicated for four SNPs in SOX10, BCL2, OPRM1, and TRPV1. The population attributable risk for each of the four SNPs ranged from 5% to 35%, with a cumulative risk of 62%. According to the multiplicative model, the odds of developing neurotoxicity increase by a factor of 1.64 for every risk genotype. Patients possessing three risk variants have an estimated OR of 4.49 (2.36–8.54) compared to individuals with 0 risk variants. Neither the four SNPs nor the risk score were associated with progression-free survival or overall survival.

Conclusions: This study shows that SNPs in four genes have a significant cumulative association with increased risk for the development of chemotherapy-induced neurotoxicity, independent of patient survival.

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Introduction

Combination chemotherapy with a platinum-containing drug (cisplatin, carboplatin, oxaliplatin) and a taxane (docetaxel, paclitaxel) have showed improved outcomes for many malignancies including ovarian and lung cancer (1–3). However, the efficacy of this therapy is often compromised because of debilitating toxicities, especially neurotoxicity. Despite initial tumor control, cancer patients treated with chemotherapy often must discontinue therapy due to peripheral neuropathy. There has also been the suggestion that presence of neuropathy may be associated with better progression-free survival in ovarian cancer (4).

There have been many attempts to circumvent or minimize the neurotoxic effects of the chemotherapeutic agents (5). Interventions using neuroprotectant agents including the administration of reduced glutathione (6), magnesium and calcium (7, 8), vitamin E (9), and xali-proden (10) are of interest, but require further studies to determine if there is a reproducible reduction of neurotoxic symptoms and no adverse effect on tumor control. In the absence of a modulator of neurotoxicity, prospective identification of the specific patients at greatest risk for this adverse event would allow informed decisions on therapy selection.

Previous studies designed to identify clinically relevant genetic factors associated with chemotherapy-induced neurotoxicity have typically explored variation in genes related to the drug disposition or other drug-related pathways (11–14). The lack of a replication strategy has limited the utility of the study results (15).

The purpose of this study was to evaluate the association of genetic variants in prespecified candidate genes with chemotherapy-induced neurotoxicity. The results of this study provide a pharmacogenetic model for predicting risk
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Translational Relevance
The ability to predict which patients are at highest risk of chemotherapy-induced peripheral neuropathy would enable clinicians to make more informed treatment decisions. This association study of credentialed candidate genes found and replicated 4 single-nucleotide polymorphisms (SNP) that can categorize an ovarian cancer patients’ risk of neuropathy when treated with platinum/taxane combination therapy. Patients possessing 3 risk variants have 4.5 times greater risk of neuropathy compared with individuals with no risk variants. The variants did not associate with overall survival. These variants are ready for confirmatory prospective studies to validate the utility in neuropathy risk stratification. Patients who are predicted to be at high risk could be offered alternative treatment options that are similarly effective but safer for those individuals.

Materials and Methods

Patients and clinical data
The Scottish Randomized Trial in Ovarian Cancer (SCOTROC1) trial randomized 1,077 patients with locally advanced or metastatic ovarian cancer to treatment with either docetaxel–carboplatin (n = 539) or paclitaxel–carboplatin (n = 538; ref. 16). Correlative science studies, including germline DNA, were prospectively obtained and available from 914 individuals (11). All patients provided written informed consent. Details of the SCOTROC1 trial (NCT00003998), patient characteristics, treatment outcomes, and toxicities for each treatment arm were previously described (16). Of particular relevance to this report, the presence of sensory neuropathy was graded before each cycle of chemotherapy, using the NCI-CTC ordinal assessment system that continues to be the standard for large oncology clinical trials. Data were not collected on the degree and timing of reversibility of the peripheral neuropathy phenotype. Patients had not received previous chemotherapy and did not have clinically detected peripheral neuropathy before the initiation of therapy.

Candidate–gene association study design
A total of 60 candidate genes were chosen from 3 categories: nerve-related genes, inherited peripheral neuropathy genes, and drug-related genes (Supplementary Table S1). These genes were derived from studies of platinum/taxane pharmacology, dorsal root ganglia biology, inherited peripheral neuropathy discovery, or genome-wide association literature conducted in human subjects (5, 17, 18). An Illumina GoldenGate custom SNP array (Illumina) with 1,536 SNPs was designed based on linkage disequilibrium, putative function, and prior literature evidence of effect on gene function (Supplementary Table S2).

Genotyping
DNA was extracted from whole blood samples obtained before the start of therapy. Genotyping of the 1,536 SNP array was conducted by the Duke University Center for Human Genetics Molecular Genetics core facility using methods that have been previously described (19) and was analyzed used the Illumina Beadstudio software. An additional 33 SNPs that were not suitable for the Illumina array were genotyped using pyrosequencing (Biotage; ref. 11). Two SNPs were assessed using Taqman Allelic Discrimination assays (Applied Biosystems) as previously described (17).

Data analysis
Patients that had less than 90% overall complete geno-type information were removed (101 patients), as were patient with a lack of neurotoxicity information (5 patients), for a final n = 808. SNPs that had less than 90% overall complete patient information or showed significant deviations from Hardy–Weinberg equilibrium (P < 0.05 based on the results of a χ2-test for independence after Bonferroni corrections) were removed (resulting in 1,261 final SNPs).

To prevent confounding due to population stratification, principle component analysis was done on these 1,261 high-quality SNPs (20). Principle component analysis confirmed the homogeneity of the SCOTROC1 patient cohort. Subsequently, logistic regression with step-wise selection was used to determine clinical covariates that were associated with neurotoxicity: Eastern Cooperative Oncology Group (ECOG) performance status, grade of gastrointestinal (GI) toxicity, and treatment arm. Time to the first cycle at which ≥ grade 2 neuropathy was experienced was included as a covariate in the discovery phase only to reduce variability and improve power to detect genetic variations associated with toxicity (no matter how long it took to experience the toxicity). Only the clinical covariates of ECOG performance status and treatment arm were included in all subsequent association analyses (Table 1); the full distributions of all clinical characteristics can be seen in Supplementary Table S3.

To assess which SNPs were associated with moderate to severe neurotoxicity, we compared individuals with moderate to severe neurotoxicity (NCI-CTC grade 2–4) to those with mild or no neurotoxicity (NCI-CTC grade 0–1) under a case/control framework. From the overall available population, test and replication datasets were created by randomization to contain 50% of the cases and 50% of the controls. Each cohort had 404 patients, with cases (grades 2/3/4 neurotoxicity) = 91 and controls (grades 0/1 neurotoxicity) = 313. All SNPs were first evaluated in the testing cohort, and those declared nominally significant were evaluated in the replication cohort. To determine single-locus association with case/control status, logistic regression was used for each SNP including important clinical covariates using genotypic encoding rather than assuming an additive genetic model. Logistic regression was first done on the test cohort, and SNPs with nominal P-values < 0.05 were
assessed in the replication cohort and were considered significant replications if $P < 0.05$ in the replication set. To further reduce potential false positive findings, only the SNPs which met these criteria and were consistent in direction of the risk effect for each genotype (positive vs. negative estimated OR) were considered true replications. For each replicating SNP, we selected the most likely genetic model for risk by fitting additive, dominant, recessive, and genotypic models and selecting the model which maximized the likelihood, or equivalently minimized the model deviance. From this model, we were able to determine the risk genotypes for each SNP.

To correct for the multiple comparisons made and to account for the possibility of false positive findings and the multistage analysis procedure, we carried out permutation testing to obtain a distribution of joint $P$-values for our initial association analysis and a distribution of $P$-values for the subsequent analysis of risk genotype score, and corrected $P$-values were calculated from these distributions. A total of 1,000 permuted datasets were generated by permuting neurotoxicity case/control status and repeated our entire analysis procedure including our two-stage association analysis, our determination of risk genotype, and analysis of risk genotype score on each of the 1,000 datasets. Only SNPs with corrected $P$-values corresponding to a family-wise error rate of 0.05 were considered statistically significant.

Once the significant SNPs and most plausible genetic model were determined, we further quantified the effect due to each SNP using population attributable risk (PAR). PAR is the proportion of the incidence of severe neurotoxicity in the population that is due to the risk genotype (the reduction in incidence of severe neurotoxicity that would be observed if the risk genotype were not present). The PAR for each SNP as well as the cumulative PAR for all significant SNPs can be calculated as follows:

$$\text{PAR}_i = \frac{p_i(\text{OR}_i - 1)}{\sum_{i=1}^n (\text{OR}_i - 1) + 1},$$

$$\text{PAR} = 1 - \left( \prod_{i=1}^n (1 - \text{PAR}_i) \right),$$

where OR$_i$ is the OR of the risk genotype for SNP $i$ and $p_i$ is the prevalence of the risk genotype for SNP $i$ (21, 22).

To explore the cumulative effect of all significant SNPs, we calculated a neurotoxicity risk genotype score for each individual which was equal to the number of risk genotypes across all significant SNPs. We fitted a logistic regression model on the number of risk genotypes, as well as the important clinical covariates to reduce possible confounding effects of the covariates on toxicity. A significant result for risk genotype score would indicate a trend in the number of risk genotypes; as number of risk genotypes increases, the odds for developing severe neurotoxicity also increase. We conducted the assessment with only the variables that would be known before the start of therapy (including ECOG status and treatment arm) to assess a clinical prediction model. The strength of the clinical prediction model was evaluated using area under the curve (AUC) analysis, comparing a model with clinical predictors only to a model also including SNPs.

We also investigated whether the individual SNPs significantly associated with neurotoxicity and the composite risk genotype score were associated with both survival time and progression-free survival time in the full cohort using a Cox proportional hazards model which included the significant clinical covariates to control for confounding. All data

| Table 1. Clinical characteristics of patients from both the discovery and replication cohorts (for significant covariates) |
|-----------------|-----------------|-----------------|
| **Variable**    | **Full data**   | **Discovery cohort** | **Replication cohort** |
|                 | **N (%)**       | **N (%)**        | **N (%)**              |
| Worst grade of sensory neuropathy |                |                 |                           |
| 0               | 285 (35.3)      | 142 (35.1)      | 143 (35.4)              |
| 1               | 341 (42.2)      | 171 (42.3)      | 170 (42.1)              |
| 2               | 135 (16.7)      | 66 (16.3)       | 69 (17.1)               |
| 3               | 44 (5.4)        | 23 (5.7)        | 21 (5.2)                |
| 4               | 3 (0.4)         | 2 (0.5)         | 1 (0.2)                 |
| Worst grade of GI toxicity |                |                 |                           |
| 0               | 37 (4.6)        | 18 (4.5)        | 19 (4.7)                |
| 1               | 212 (26.2)      | 116 (28.7)      | 96 (23.8)               |
| 2               | 416 (51.5)      | 201 (49.8)      | 215 (53.2)              |
| 3               | 130 (16.1)      | 62 (15.3)       | 68 (16.8)               |
| 4               | 13 (1.6)        | 7 (1.7)         | 6 (1.5)                 |
| ECOG performance status |                |                 |                           |
| 0               | 268 (32.2)      | 119 (29.5)      | 149 (36.9)              |
| 1               | 426 (52.7)      | 221 (54.7)      | 205 (50.7)              |
| 2               | 114 (14.1)      | 64 (15.8)       | 50 (12.4)               |
| Treatment       |                 |                 |                           |
| Paclitaxel/carboplatin | 400 (49.5) | 206 (51.0) | 194 (48.0) |
| Docetaxel/carboplatin | 408 (50.5) | 198 (49.0) | 210 (52.0) |
analyses were conducted in R-software (http://www.r-project.org/).

Results

ECOG performance status (OR = 1.42, P = 0.006) and grade of GI toxicity (OR = 1.42, P = 0.001) were associated with increased risk of grade 2+ neurotoxicity, and docetaxel treatment (OR = 0.30, P = 7E-11) was associated with reduced risk of grade 2+ neurotoxicity, independently of SNP associations (see Table 1 and Supplementary Table 3 for distributions of clinical characteristics).

From the 1,261 SNPs which passed quality control standards, 69 SNPs were significantly associated with grade 2+ neurotoxicity in the test patient cohort at the P < 0.05 level (Fig. 1 and Supplementary Table S4). Of those significant in the test cohort, 5 were also significant in the replication cohort. Four of these SNPs were consistent in the direction of clinical effect in the independent test and replication analyses (Table 2). The variants were in BCL2, SOX10, OPRM1, and TRPV1. All 4 SNPs reside on different chromosome, and there was no long-range linkage disequilibrium between these 4 SNPs, indicating that these are

Table 2. SNPs significantly associated with moderate to severe neurotoxicity in the replication cohort

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>Base change</th>
<th>Chromosome</th>
<th>Position</th>
<th>MAF</th>
<th>Permutation corrected P-value</th>
<th>OR (95% CI)</th>
<th>Risk genotype</th>
<th>Proportion cases per genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs139887</td>
<td>SOX10</td>
<td>C→G</td>
<td>22</td>
<td>38371396</td>
<td>0.38</td>
<td>0.001</td>
<td>1.77 (1.21, 2.59)</td>
<td>CG</td>
<td>CC = 24/111 = 0.22</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CG = 98/355 = 0.28</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>GG</td>
<td>CG = 58/320 = 0.18</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AG</td>
<td>AA = 10/26 = 0.38</td>
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<td></td>
<td></td>
<td></td>
<td>GG</td>
<td>AA = 49/272 = 0.18</td>
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<td></td>
<td></td>
<td>AG</td>
<td>AA = 123/506 = 0.24</td>
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<td></td>
<td>GG</td>
<td>AA = 157/653 = 0.24</td>
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<td></td>
<td>AG</td>
<td>AA = 23/144 = 0.16</td>
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<td></td>
<td></td>
<td>GG</td>
<td>AA = 2/10 = 0.20</td>
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<td></td>
<td></td>
<td></td>
<td>AG</td>
<td>AA = 57/314 = 0.18</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>GG</td>
<td>AG = 103/383 = 0.27</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AG</td>
<td>AG = 22/86 = 0.20</td>
</tr>
</tbody>
</table>

Estimates are adjusted for ECOG performance status and treatment status.
The addition of the genotype risk score improved the strength of our predictive model, measured by the AUC with clinical predictors only (AUC = 0.67 vs. 0.71 in the full cohort); it should be noted that our risk predictive model relies on the assumption that all 4 SNPs have equal effects, which may not be the case. To assess this assumption, we investigated a model where the score was weighted by the OR of each SNP (which was wild-type homozygous in 2 cases and heterozygous in 2 cases) optimally describes the true nature of the genotype–phenotype relationship or if the use of an assumed genetic model could outperform this system.

None of the 4 significant SNPs were associated with progression-free survival or overall survival. This decouples the theoretical link between neurotoxicity and tumor control and does not support the worry that reducing neurotoxicity risk might also reduce anticancer effect. The unlinking of neurotoxicity and response was also recently corroborated in breast cancer (23). Indeed, this data suggests that intervention or prevention strategies for platinum/taxane neurotoxicity based on these 4 genetic variants are not likely to adversely influence the efficacy of the chemotherapeutic agent. Knowledge of genetic risk may serve as a valuable tool in deciding whether to use chemotherapy, to determine if patients may be at risk for neurotoxicity, to modify regimens to minimize neurotoxicity, or to consider these factors in the counseling of patients with advanced disease, where the goal is palliation of the patient and risk of severe neurotoxicity may be detrimental.

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prediction tool so that a patient’s level of acceptance of risk can be calibrated with approximate level of risk of neurotoxicity based on the number of risk genotypes.

Although the overall goal of this study was discovery and validation of genetic associations, several interesting covariates that would not be known before the treatment, and could not be used in a predictive model, such as time to first cycle of grade 2 neurotoxicity and grade of gastrointestinal toxicity were identified. These endpoints likely reflect underlying biological risk that is not captured by the variants assessed in this study. Analysis of the variants with only treatment arm as a covariate yielded similar results, with similar effect size estimates and directions, and similar conclusions regarding association with toxicity and a lack of association with overall survival. This supports future work in additional cohorts to focus on testing and refining such a predictive pharmacogenomics model.

*BCL2, SOX10, OPRM1, and TRPV1* encompass a broad spectrum of functionalities including a role in apoptosis, a transcription factor, an opioid receptor, and therapeutic targets of pain management, respectively. These genes were among 58 candidate genes, all with biologic plausibility for impact on chemotherapy-induced peripheral neuropathy in humans. However, the mechanism by which the SNPs or genes we identified influences the development of neurotoxicity is yet to be defined. The variants identified in this study are not known to have direct functional consequence, as those in *BCL2, SOX10, and OPRM1* are intronic and the *TRPV1* variant lies outside of the coding region. In addition, because these SNPs were chosen as “tagging” SNPs based on the linkage disequilibrium structure, it is likely that these SNPs are not directly responsible for biological effect, but act as markers for the important genomic regions. Future studies should try to refine these association signals through more fine mapping. For example, TRPV family members have recently been proposed to have effects on chemotherapy-associated neurotoxicity via glutathione pathway modulation, and using data from Caucasian individuals from

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**Table 5.** Association between risk genotype and risk of neurotoxicity

<table>
<thead>
<tr>
<th>Genotype risk score</th>
<th>Proportion of cases with risk genotype</th>
<th>Proportion of controls with risk genotype</th>
<th>Model with only a priori covariates OR</th>
<th>95% CI for a priori covariate model</th>
<th>Permutation corrected P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.06</td>
<td>0.94</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1</td>
<td>0.17</td>
<td>0.83</td>
<td>1.64</td>
<td>(1.3313, 2.0442)</td>
<td>0.007</td>
</tr>
<tr>
<td>2</td>
<td>0.26</td>
<td>0.74</td>
<td>2.72</td>
<td>(1.7724, 4.1789)</td>
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<tr>
<td>3</td>
<td>0.31</td>
<td>0.69</td>
<td>4.49</td>
<td>(2.3597, 8.5427)</td>
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</tr>
<tr>
<td>4</td>
<td>0.75</td>
<td>0.25</td>
<td>7.41</td>
<td>(3.1415, 17.4632)</td>
<td>–</td>
</tr>
</tbody>
</table>

*Both ECOG performance status and treatment arm were used as covariates and were determined to be associated with neurotoxicity. Neurotoxicity risk increases per ECOG performance category (OR = 1.45; 95% CI, 1.11–1.89; uncorrected P = 0.00631) and patients undergoing docetaxel–carboplatin treatment were at a reduced risk of developing neurotoxicity (OR = 0.30; 95% CI, 0.21–0.43; uncorrected P = 8 × 10⁻¹⁷).*

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the HapMap, we know that the SNP in TRPV1 is in strong linkage disequilibrium \( r^2 > 0.8 \) with several expression quantitative trait loci (according to data provided in SCANdb; www.scandb.org; refs. 24, 25). Although functional understanding is desired, the replication of these SNPs in this study prepares the way for clinical intervention studies.

All patients in this study were treated with either docetaxel–carboplatin or paclitaxel–carboplatin combination therapy, with approximately equal numbers randomized to both the discovery and replication cohorts; however, paclitaxel and docetaxel may have different genetic markers of neurotoxicity. To evaluate this possibility, we separately examined each of the 4 SNPs stratified by treatment group, which yielded similar results (although less stable due to the reduced sample size), suggesting that the identified SNPs are general markers of platinum/taxane-induced neurotoxicity. Furthermore, because our full patient cohort was treated with combination platinum/taxane chemotherapy and both drug classes independently have been shown to cause neurotoxicity, it is possible that the neurotoxicity could result from either or both of the drugs. The genes identified in this study are not unique to the mechanism of action of either platinum (DNA adduct formation) or taxanes (inhibit microtubule dynamics), therefore, it cannot be assumed that this genetic risk model for neurotoxicity will be predictive in other contexts. Indeed, the recently replicated associations of FGD4 and CYP2C8*3 with paclitaxel-associated neurotoxicity (17, 26) were not observed in SCOTROC1 patients receiving the combination of carboplatin and paclitaxel, suggesting that predictive biomarkers may be unique to the drug combinations being deployed (11).

Several limitations of this post hoc analysis of a completed clinical study should be considered. The primary objective of the SCOTROC1 trial was not to assess peripheral neuropathy, and thus some potentially useful data are not as thoroughly established, including baseline incidence of neuropathy or other known clinical risk factors such as diabetes. Also, the neuropathy phenotype was collected exclusively by clinician reporting using the standard NCI-CTC scale. Although this scale is still the industry standard for international cancer clinical trials, more sensitive scales that incorporate patient assessment have been developed to overcome this challenge (27). Objective neuropathy measurement using nerve conduction or other surrogate metrics may be an even more sensitive technique for collecting this phenotype in future studies, should these methods be refined to use in the context of routine oncology practice. Unfortunately it is extraordinarily difficult and expensive to prospectively collect comprehensive toxicity data in large multicenter clinical trials and impossible to collect it retrospectively.

This study is a first step in defining a pharmacogenetic risk model for this treatment-limiting side effect of platinum–taxane chemotherapy (28). This gives a basis for studies that further validate this risk model in independent patient cohorts to identify the power of this model to predict chemotherapy-induced neurotoxicity as well as to evaluate the utility of these genes as therapeutic targets.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: S. McWhinney-Glass, R. Brown, A.A. Motsinger-Reif, H.L. McLeod
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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.I. Winham, D.L. Hertz, A.A. Motsinger-Reif, H.L. McLeod
Writing, review, and/or revision of the manuscript: S. McWhinney-Glass, S.I. Winham, D.L. Hertz, J. Paul, Y. He, R. Brown, A.A. Motsinger-Reif, H.L. McLeod
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References


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