

Gemcitabine and Platinum Pathway Pharmacogenetics in Asian Breast Cancer Patients

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Abstract. *Background/Aim:* Gemcitabine/carboplatin is efficacious in breast cancer but results in significant hematologic toxicities. We employed a multi-gene approach to identify variants to predict its toxicities. *Patients and Methods:* Twenty-six gemcitabine and platinum-based DNA repair pathway polymorphisms were correlated with gemcitabine pharmacokinetics, hematologic toxicities, response and survival in 41 Asian breast cancer patients receiving gemcitabine/carboplatin. *Results:* The combined effects of solute carrier family (*SLC*)28A1+1528C>T and thymidylate synthetase (*TYMS*)+1494del6 significantly influenced hematologic toxicities: 89% of patients who possessed either *SLC*28A1+1528TT or *TYMS*+1494ins6/ins6 (n=9) developed grade 4 thrombocytopenia, versus 14% with neither genotype (n=29; p<0.001). In concordance, all patients who possessed either genotype developed grade 3/4 neutropenia, compared to 38% with neither genotype (p=0.001). None of the other genetic variants analyzed correlated with drug pharmacokinetics and pharmacodynamics. *Conclusion:* Approximately one-quarter of our Asian cohort carried *SLC*28A1+1528TT or *TYMS*+1494ins6/ins6, which are associated with increased myelotoxicity from gemcitabine/carboplatin. This has potential utility in treatment selection and genotype-based dosing strategies.

Gemcitabine combined with carboplatin is commonly used in the treatment of advanced breast cancer and non-small cell lung cancer (NSCLC). Myelosuppression, particularly thrombocytopenia, is a frequent dose-limiting toxicity which demonstrates significant inter-individual variability, and may

be influenced by genetic polymorphisms. To establish the correlation between genotype and drug pharmacokinetics and pharmacodynamics, we studied 32 single-nucleotide polymorphisms (SNPs) from the gemcitabine pharmacologic and platinum-based DNA repair pathways in a prospective clinical trial of 41 patients with advanced breast cancer treated with gemcitabine/carboplatin.

Patients and Methods

The study population comprised females aged >18 years, with histologically confirmed metastatic breast cancer and adequate organ function, who had received prior anthracyclines and taxanes. Patients received intravenous gemcitabine 1000 mg/m² on days 1 and 8, and carboplatin (area under the curve [AUC] of 5) on day 1 of a 21-day cycle, for a maximum of 6 cycles. Response was evaluated using the RECIST criteria (1). The objective response rate (ORR) was defined as the percentage of patients achieving complete response (CR) or partial response (PR); the clinical benefit rate (CBR) was defined as the percentage of patients achieving CR, PR or stable disease (SD) maintained for at least 3 months. Toxicity assessments were in accordance with National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (2). Hematologic parameters were monitored on days 8 and 15, and within 4 days prior to the initiation of each cycle (3).

DNA extraction and genotyping. Germline DNA was extracted from peripheral mononuclear cells using the Gentra DNA Purification kit (Gentra Systems, Minneapolis, MN, USA). Gene variants were selected based on the following: SNPs that i) have been identified as potentially clinically or functionally significant in previous publications, ii) encode a non-synonymous change and iii) have reported allelic frequencies of >5%. These included 26 variants from 10 gemcitabine pharmacologic pathway genes encoding enzymes involved in transport [solute carrier family (*SLC*)28A1+419(ins), *SLC*28A1+565G>A, *SLC*28A1+709C>A, *SLC*28A1+1368G>A, *SLC*28A1+1528C>T, *SLC*28A1+1561G>A; *SLC*28A2+65C>T, *SLC*28A2+225C>A; *SLC*28A3+338A>G; *SLC*29A1+600G>A], metabolism [cytidine deaminase (*CDA*)+79A>C, *CDA*+208G>A, *CDA*+435C>T; deoxycytidine kinase (*DCK*)IVS-1110A>G, *DCK*+2190A>G; deoxycytidine monophosphate deaminase (*DCTD*)+315T>C] and activity [ribonucleotide reductase M1

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(*RRM1*)-524T>C, *RRM1*-37C>A, *RRM1*+17G>A, *RRM1*+536A>C, *RRM1*+589T>G, *RRM1*+2333T>C; thymidylate synthetase (*TYMS*) T589T>G, *TYMS*-58G>C, *TYMS*+1494del6 and polymerase alpha 2 (*POLA2*)+1747G>A]. All but *POLA* and *TYMS* have demonstrated functional activity related to gemcitabine in pre-clinical studies (4-6); *SLC28A1*, *CDA* and *RRM1* gene variants have been reported to influence gemcitabine pharmacodynamics in clinical studies (4, 6, 7); *TYMS* is inhibited by the gemcitabine monophosphate deamination product, resulting in impaired DNA synthesis (5), and *TYMS* promoter and 3'-untranslated region polymorphisms correlated with 5-fluorouracil (5-FU) pharmacodynamics in colorectal cancer patients (8, 9), and may be similarly implicated in gemcitabine pharmacogenetics. In addition, we selected six variants in three nucleotide excision repair pathway enzyme genes that were associated with clinical outcomes in platinum-based chemotherapy (10, 11): excision repair cross-complementing group 1 (*ERCC1*)+118C>T, *ERCC1*+8092C>A, xeroderma pigmentosum group D (*XPD*)+156C>A, *XPD*+312G>A, *XPD*+751A>C, and x-ray repair cross-complementing group 3 (*XRCC3*)+241C>T. Genotyping was performed using pyrosequencing or comprehensive sequencing as previously described (4, 12). Designation of the wild-type genotype was in accordance with the NCBI database (13), and only SNPs that were polymorphic in the cohort were included in subsequent correlative analyses.

Gemcitabine pharmacokinetic analysis. Blood samples were collected before initiation of a 30 min gemcitabine infusion, and at 10, 20, 30, 60 and 120 min after the end of the infusion on day 1 of cycle 1. Intracellular 2',2'-difluorodeoxycytidine triphosphate (dFdCTP) concentrations were determined through an ion-exchange HPLC method (14). Non-compartmental analysis was performed using Win Nonlin5.2. (Pharsight Corporation, California, CA, USA) to calculate the major pharmacokinetic parameters: clearance, half-life of the terminal disposition phase, and volume of distribution. AUC was estimated using the log-linear trapezoidal option from time 0 to infinity.

Statistical analysis. Associations between genotype and tumor response were assessed by Chi-square testing, while mean values of hematologic parameters were compared using Student's *t*-test. Non-parametric tests were used as appropriate. Kaplan-Meier methods and log-rank tests were used to assess progression-free (PFS) and overall survival (OS). A *p*-value <0.002 was considered statistically significant after correction for multiple testing of the 26 polymorphic genetic variants that were analyzed. Statistical tests were two-sided and performed using SPSS software version 14.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics. Forty-one patients were enrolled; their mean age was 52 (range, 33-67) years and they received a median of 4 chemotherapy cycles (range, 1-6). The ORR was 38%, while the CBR was 57%. The incidence of grade 4 neutropenia, anemia and thrombocytopenia on day 15 of cycle 1 was 13%, 0% and 32% respectively; 15% developed febrile neutropenia, while 7% required platelet transfusions. Treatment delays were experienced by 56% due to prolonged hematologic toxicity, 56% required dose reductions of

gemcitabine, and 10% were withdrawn from the study because they exceeded the permissible two gemcitabine dose reductions. Twenty-six percent received two or more prior lines of palliative chemotherapy, but these patients did not experience more hematologic toxicities compared to those who received 0-1 prior lines of palliative chemotherapy (day 15 cycle 1 grade 4 thrombocytopenia 20% vs. 35.7%, *p*=0.36; day 15 cycle 1 grade 3-4 neutropenia 50% vs. 53.6%, *p*=0.85, respectively). The median duration of follow-up was 9.9 months, and at the time of analysis, all evaluable patients experienced disease progression and 37 had died. The median PFS was 4.6 months (95% CI: 3.3-5.9 months), and the median OS was 10.2 months (95% CI: 9.4-11.0 months).

Genotype data. Thirty-two variants in 13 candidate genes were genotyped (Table I). As no sequence variation was identified at 6 gene loci in the first 22 individuals genotyped (*CDA*+208G>A, *RRM1*+17G>A, *RRM1*+536A>C, *RRM1*+589T>G, *RRM1*+2333T>C, *SLC29A1*+600G>A), further genotyping and correlative analyses were not performed for these loci, and only 26 SNPs were correlated with drug pharmacokinetics and pharmacodynamics. All gene frequencies were in Hardy-Weinberg equilibrium.

Correlation of genotypes with hematologic toxicity. Two gemcitabine pathway SNPs, *SLC28A1*+1528C>T and *TYMS*+1494del6, were associated with hematologic toxicities. Patients with *SLC28A1*+1528 variant TT genotype (*n*=6) had higher rates of grade 4 thrombocytopenia (83 vs. 22%, TT vs. CC/CT, *p*=0.008) and grade 3-4 neutropenia (100 vs. 44%, TT vs. CC/CT, *p*=0.021) on day 15 cycle 1. Those with *TYMS*+1494ins6/ins6 genotype (*n*=3) also experienced higher incidence of grade 4 thrombocytopenia at day 15 of cycle 1 (100 vs. 26%, ins6/ins6 vs. non-ins6/ins6, *p*=0.026). No patient carried both *SLC28A1*+1528TT and *TYMS*+1494ins6/ins6 genotypes. When these variants were analyzed in combination, 89% of those who possessed either the *SLC28A1*+1528TT or *TYMS*+1494ins6/ins6 genotype (*n*=9) developed grade 4 thrombocytopenia, compared to 14% of those with neither genotype (*n*=29; *p*<0.001). In concordance, 100% of patients who possessed either *SLC28A1*+1528TT or *TYMS*+1494ins6/ins6 developed grade 3-4 neutropenia, compared to 38% of those with neither genotype (*p*=0.001). No significant correlation was found between the other gemcitabine or DNA repair pathway genetic variants with hematologic toxicities. There was also no association between genotype and gemcitabine pharmacokinetics, or with tumor response and survival.

Discussion

In this Asian breast cancer study, the combination of gemcitabine and carboplatin was efficacious but resulted in substantial hematologic toxicities. By screening 32 SNPs

Table I. Summary of genetic variants and their frequencies in the study population.

Gene variants (rs number ^a /reference)	Amino acid change	Genotype frequencies (%)	Frequency of variant allele (q)
<i>SLC28A1</i> +419 (rs17215836)	Insertion	II:ID:DD=7:27:66	0.80
<i>SLC28A1</i> +565 G>A (rs2290272)	V189I	GG:GA:AA=35:58:7	0.35
<i>SLC28A1</i> +709 C>A (rs8187758)	Q237K	CC:CA:AA=42:56:2	0.30
<i>SLC28A1</i> +1368 G>A (rs2242048)	Q456Q	GG:GA=95:5	0.03
<i>SLC28A1</i> +1528 C>T (rs2242047)	R510C	CC:CT:TT=44:39:17	0.37
<i>SLC28A1</i> +1561 G>A (rs2242046)	N521D	GG:GA=83:17	0.09
<i>SLC28A2</i> +65 C>T (rs11854484)	P22L	CC:CT:TT=64:34:2	0.19
<i>SLC28A2</i> +225 C>A (rs1060896)	R75S	CC:CA:AA=56:37:7	0.26
<i>SLC28A3</i> +338 A>G (rs10868138)	Y113C	AA:AG=74:24	0.12
<i>SLC29A1</i> +600 G>A ^d (rs1128870)	E200E	GG=100	0
<i>CDA</i> +79 A>C (rs2072671)	K27Q	AA:AC:CC=71:27:2	0.16
<i>CDA</i> +208 G>A ^d (rs60369023)	A70T	GG=100	0
<i>CDA</i> +435 C>T (rs1048977)	T145T	CC:CT:TT=56:39:5	0.25
<i>DCK</i> IVS-1110 A>G (rs3775289)	Intron 1	AG:GG=7:93	0.96
<i>DCK</i> +2190 A>G ^{b,d} (rs00011480)	3'-UTR	AA=100	0
<i>DCTD</i> +315 T>C ^c	V105V	TT:TC:CC=66:29:5	0.20
<i>RRM1</i> -524 T>C (rs11030918)	Promoter	TT:TC:CC=36:50:14	0.39
<i>RRM1</i> -37 C>A (rs12806698)	Promoter	CC:CA:AA=35:52:13	0.39
<i>RRM1</i> +17 G>A ^d (rs2584879)	R6Q	GG=100	0
<i>RRM1</i> +536 A>C ^d (rs12808005)	H179P	AA=100	0
<i>RRM1</i> +589 T>G ^d (rs12789346)	W197G	TT=100	0
<i>RRM1</i> +2333 T>C ^d (rs2229196)	V778A	TT=100	0
<i>TYMS</i> TSER/ -58 G>C ^c (rs34743033)	5'UTR	Functional 2R (2R/2R, 2R/3C, 3C/3C): 32 Functional 3R (2R/3G, 3G/3C, 3G/3G): 68	0.68 (3R)
<i>TYMS</i> +1494del6 (rs34489327)	3'UTR	Ins6/Ins6:Ins6/Del6/Del6/Del6=10:46:44	0.67 (del6)
<i>POLA2</i> +1747 G>A (rs487989)	G583R	GG:GA:AA=85:13:2	0.09
<i>ERCC1</i> +118 C>T (rs11615)	N118N	CC:CT:TT=63:29:7	0.22
<i>ERCC1</i> +8092 C>A (rs3212986)	3'UTR	CC:CA:AA=34:54:12	0.39
<i>XPB</i> +156 C>A (rs238406)	R156R	CC:CA:AA=20:46:34	0.57
<i>XPB</i> +312 G>A (rs1799793)	D312N	GG:GA=88:12	0.06
<i>XPB</i> +751 A>C (rs13181)	K751Q	AA:AC=83:17	0.09
<i>XRCC3</i> +241 C>T (rs861539)	T241M	CC:CT=83:17	0.09

^aNCBI SNP database; ^bCGAP <http://lpgws.nci.nih.gov/perl/snpbr>; ^cHGVBase <http://hgvsbase.cgb.ki.se>; ^dgenotypes without sequence variation; ^en=38, functional 2R/3R genotypes.

involved in gemcitabine pharmacologic and DNA repair pathways, we found that combined effects of *SLC28A1*+1528C>T and *TYMS*+1494del6 significantly influenced hematologic toxicities. Rates of grade 4 thrombocytopenia were more than 6-fold higher and grade 3-4 neutropenia almost 3-fold higher in individuals possessing either *SLC28A1*+1528TT or *TYMS*+1494ins6/ins6, compared to those possessing neither genotype. Our findings were consistent with those of another Asian study of gemcitabine/carboplatin in NSCLC patients, where linkage disequilibrium was found between *SLC28A1*+1528C>T and *SLC28A1*+1561G>A, and individuals possessing the variant *SLC28A1*+1561AA genotype experienced more severe neutropenia and thrombocytopenia. The postulated mechanism was enhanced intracellular influx of gemcitabine due to greater *SLC28A1* transporter activity (4). On the other hand,

the finding of a *TYMS* polymorphism influencing gemcitabine pharmacodynamics is novel. *TYMS*+1494ins6 allele, when analyzed as a haplotype with *TYMS* promoter region 2R polymorphism, was associated with increased 5FU-related toxicities, possibly due to reduced *TYMS* gene expression and impaired ability to protect normal cells against cytotoxic effects of *TYMS* inhibition (9). As *TYMS* is inhibited by the gemcitabine monophosphate deamination product, resulting in impaired DNA synthesis (5), a similar mechanism may account for our observation of increased gemcitabine-induced cytopenia in individuals with the *TYMS*+1494ins6/ins6 genotype, although confirmation is warranted with gene expression studies.

Pharmacogenetic differences may contribute to the well-documented inter-ethnic variability in treatment outcomes between Asians and Caucasians receiving identical

chemotherapy (15). Asians seem more susceptible to the hematologic toxicities of gemcitabine/carboplatin than Caucasians (3); although our study population was heavily pre-treated, hematologic toxicities did not appear to be related to the number of prior chemotherapy regimens. Genotypes associated with myelotoxicity, *SLC28A1*+1528TT or *TYMS*+1494ins6/ins6, were common in our population, with approximately one-quarter possessing either genotype. Interestingly, the literature reports inter-ethnic differences in frequency distribution of these variant alleles. *SLC28A1*+1528T allele is present in approximately 40% of Asians (37% in our cohort, 43% in Asian Americans), compared to only 0.5% of Caucasians and 4.5% of African Americans (16), potentially contributing to the higher incidence of myelosuppression observed in Asians. In contrast, the frequency of *TYMS*+1494ins6 was 33% in our cohort, concordant with that reported in another Chinese population (30%) (17), but significantly lower than that reported in Caucasians (71%,74%) (8, 18), suggesting that *SLC28A1*+1528T may be the more important of the two alleles in gemcitabine-induced myelosuppression in Asians.

Despite employing a multi-candidate-gene approach, we found no significant correlation between the majority of the variants analyzed and gemcitabine pharmacokinetics or other pharmacodynamic parameters. This may be due to small sample size, low frequency of certain variant alleles in our population, or true lack of biological significance of these variants.

Conclusion

Gemcitabine/carboplatin is active in advanced breast cancer but results in severe myelotoxicity in approximately one-fifth of Asian patients. One-quarter of our cohort possessed the *SLC28A1*+1528TT or *TYMS*+1494ins6/ins6 genotype, and were at greater than 85% risk of severe hematologic toxicities. *SLC28A1*+1528T allele is more common in Asians than Caucasians, and may contribute to the greater myelosuppression observed in Asians in response to gemcitabine/carboplatin. These findings are hypothesis-generating and with further validation, have potential clinical relevance in guiding the selection and dosing optimization of this regimen.

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