



ABCG2 C421A 다형성이 만성 골수성 백혈병 환자의 imatinib 치료에 미치는 영향: 체계적 문헌고찰 및 메타분석

오다현 · 천부순*

인제대학교 약학대학

(2016년 2월 25일 접수 · 2016년 3월 9일 수정 · 2016년 3월 11일 승인)

ABCG2 C421A Polymorphism and Imatinib Response in Chronic Myeloid Leukemia: A Systematic Review and Meta-Analysis

Da Hyun Oh and Pusoon Chun*

College of Pharmacy, Inje University, 197 Inje-ro, Gimhae, Gyeongnam 50834, Republic of Korea

(Received February 25 2016 · Revised March 9, 2016 · Accepted March 11, 2016)

ABSTRACT

Objective: To estimate the association between ABCG2 C421A polymorphism and response to imatinib in chronic myeloid leukemia.

Methods: A systematic review was conducted to evaluate the effect of ABCG2 C421A polymorphism on imatinib response. The databases of PubMed, Embase, Web of science, CINAHL with FullText, and Cochrane Library were searched for all published studies from inception to December 2015. The following terms were used with functions of 'AND' and 'OR': 'chronic myeloid leukemia', 'CML', 'drug transporter', 'ABCG2', 'BCRP', 'polymorphisms', 'SNPs', and 'imatinib'. The studies reporting the association between ABCG2 polymorphism and imatinib response were evaluated. **Results:** A total of 7 studies were included in the present meta-analysis. The pooled analysis showed that ABCG2 c.421CC genotype was significantly associated with poor response to imatinib under the dominant model (CC vs CA+AA; OR: 0.56; 95% CI: 0.41, 0.77; $p = 0.0004$). The subgroup analysis of Asian studies demonstrated a significantly lower response in c.421CC genotype than in c.421CA or c.421AA genotype (OR: 0.52; 95% CI: 0.37, 0.73; $p = 0.0002$). In subgroup analyses of 5 studies, the patients with the c.421CC genotype exhibited higher risk for worse response than the patients with c.421CA or c.421AA genotype (heterozygote codominant model: CC vs. AC; OR: 0.49, 95% CI: 0.33, 0.73; $p = 0.0006$; homozygote codominant model: CC vs AA; OR: 0.43; 95% CI: 0.25, 0.75, $p = 0.003$). **Conclusion:** The ABCG2 c.421CC genotype was significantly associated with poor response to imatinib compared to the c.421CA and c.421AA genotypes in chronic myeloid leukemia, especially in Asian patients.

KEY WORDS: ABCG2 polymorphism, chronic myeloid leukemia, imatinib, response

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterized by increased proliferation of the granulocytic cell line. The hallmark genetic abnormality of CML is the Philadelphia chromosome arising from a reciprocal translocation between chromosomes 9 and 22. This translocation generates BCR-ABL, a constitutively active tyrosine kinase that plays a critical role in the pathogenesis of CML.^{1,2)}

Imatinib is an orally administered protein-tyrosine kinase inhibitor. It is recommended as first-line treatment for people

with Philadelphia-chromosome-positive (Ph+) CML in the chronic phase.^{3,4)} Imatinib inhibits BCR-ABL by occupying the ABL domain adenosine triphosphate (ATP)-binding site. This maintains BCR-ABL in an inactive conformation, thereby preventing substrate phosphorylation and downstream activation of leukemogenic signal transduction.^{5,6)} Imatinib has also been shown to be a substrate and inhibitor of ABCG2.⁷⁻⁹⁾ ABCG2, also known as breast cancer resistance protein (BCRP), is the second member of the G family of ATP-binding cassette

*Correspondence to: Pusoon Chun, College of Pharmacy, Inje University, 197 Inje-ro, Gimhae, Gyeongnam 50834, Republic of Korea
Tel: +82-55-320-3886 (+82-10-3245-5683), Fax: +82-55-320-3940
E-mail: pusoon@inje.ac.kr

(ABC) transporters, which pumps a wide range of molecules out of cells in an energy-dependent manner. ABCG2 is expressed at high levels in the Blood-Brain Barrier (BBB), gastrointestinal tract, kidney, and liver. Since ABCG2 is mostly found on the apical surface of cells,¹⁰⁾ it is thought to play an important role in preventing intestinal absorption and in mediating excretion of its substrates, including tyrosine kinase inhibitors.¹¹⁾ High ABCG2 expression has also been found in a variety of solid tumors and in hematologic malignancies.^{12,13)}

Although, imatinib has greatly improved clinical outcomes and prognosis for CML patients, a proportion of patients show suboptimal response or develop resistance to the drug.¹⁴⁾ Mounting evidence has demonstrated that single nucleotide polymorphisms (SNPs) modify the transporter activity of ABCG2 and affect response in patients receiving imatinib. One of the most extensively studied ABCG2 SNPs is C421A (Q141K, rs2231142), which results in a substitution of lysine for glutamine in the ABCG2 protein. Recently, Skoglund *et al.* found that the ABCG2 C421A variant exhibited significantly lower expression of ABCG2 and increased the efficacy of imatinib, as compared to homozygous wild-type in CML cell line.¹⁵⁾ Meanwhile, other studies reported that there were no significant differences in pharmacokinetic parameters such as intracellular concentrations, plasma concentrations, and clearance of imatinib among the ABCG2 polymorphisms in CML patients.^{16,17)} Considering these findings, it is necessary to clarify the association between ABCG2 C421A and response to imatinib in patients with CML. A meta-analysis was performed to determine whether the ABCG2 C421A might be useful for predicting response to imatinib in CML patients.

Materials and Methods

Literature search

A systematic literature search was performed to identify publications addressing the association between ABCG2 polymorphisms and response to imatinib in patients with CML. The databases of PubMed, Embase, Web of science, CINAHL with FullText, and Cochrane Library were searched for all published studies from inception to December 2015. The following terms were used with functions of 'AND' and 'OR': 'chronic myeloid leukemia', 'CML', 'drug transporter', 'ABCG2', 'BCRP', 'polymorphisms', 'SNPs', and 'imatinib'. The reference lists of the identified articles were hand-searched for additional pertinent publications.

Study selection and data extraction

Two investigators (PC and DHO) independently identified relevant studies and extracted detailed information from each study. Discrepancies were resolved by discussion and consensus.

To be included, original studies needed to fulfill the following criteria: (1) investigating the impact of ABCG2 SNPs on imatinib response in CML; (2) reporting sufficient data for estimating an odds ratio (OR); (3) identifying response criteria

Articles were excluded in accordance with the following criteria: (1) reviews, conference abstract or paper, editorial, and letter; (2) studies without clinical outcomes; (3) studies without genotype frequency for OR.

The extracted information included the first author's name, year of publication, country of study population, disease phase of subjects, dose of imatinib, follow-up period, response criteria, and the genotype frequency in responsive and resistant groups.

Quality assessment

The quality of each included study was assessed on the basis of the 9-star Newcastle-Ottawa Scale (NOS).¹⁸⁾ The NOS assigns up to a maximum of 9 points for the least risk of bias: 4 for selection, 2 for comparability, and 3 for assessment of outcomes (for cohort study) or exposures (for case-control study). Studies with points of 0-3, 4-6, 7-9 were considered as low, moderate and high quality, respectively.¹⁹⁾ Two researchers (PC and DHO) independently assessed the quality of the studies, and then any disagreements were resolved by discussion and consensus.

Data synthesis and analysis

The Review Manager 5, the Cochrane Collaboration software, was used for the present meta-analysis. The data from the included studies were pooled and weighted by a fixed-effects model. Pooled OR and 95% CIs were used to measure the association between the ABCG2 C421A polymorphism and imatinib response under dominant model (CC vs. CA/AA), heterozygote codominant model (CC vs. CA), and homozygote codominant model (CC vs. AA). Study heterogeneity was estimated with I^2 statistic, with values of 25%, 50%, and 75% representing low, moderate, and high degrees of heterogeneity. Two-sided P values less than 0.05 were considered statistically significant. Publication bias was evaluated by visual inspection of a funnel plot.

Results

The process of study selection is outlined in Fig. 1. Of the 428 records identified in the database search, 45 articles were considered potentially relevant. After further review of the full text of 46 studies, including 1 study identified through the reference lists, 7 case-control studies met the inclusion and

exclusion criteria of the present meta-analysis. When assessing quality of the studies, all the studies met the criteria of high quality based on NOS: 7 stars for 2 studies^{20,21)} and 8 stars for 5 studies.²²⁻²⁶⁾

Table 1 describes the main characteristics of the included studies and genotype frequencies in CML patients according to their response to imatinib treatment. A total of 735 patients

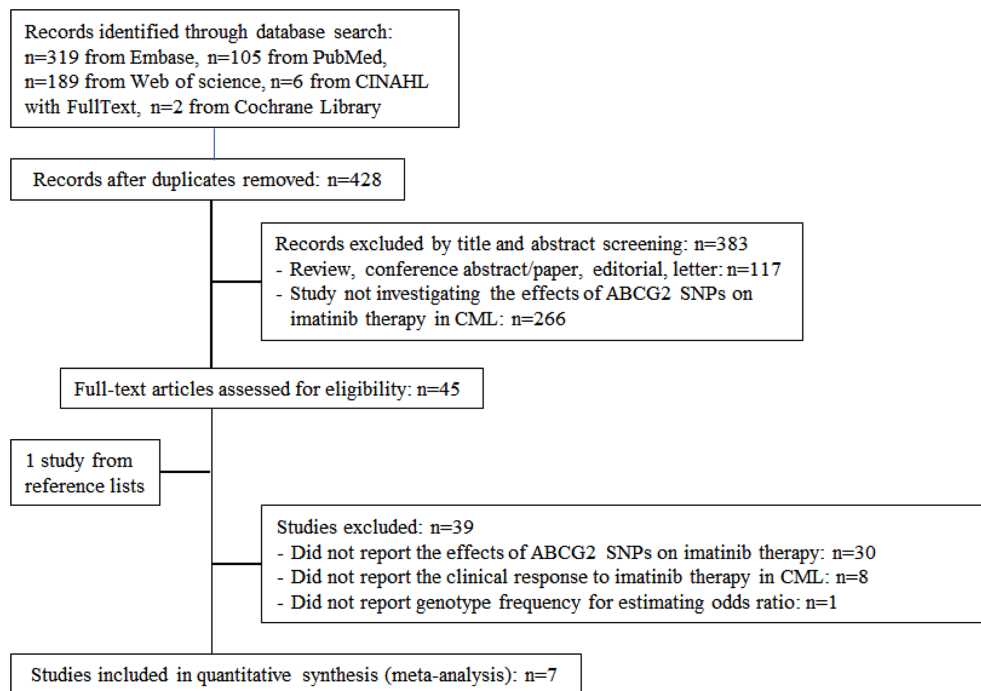


Fig. 1. Flowchart of the study selection process.

Table 1. Characteristics of the included studies and genotype frequencies according to imatinib response.

Study	Country	CML phase	Treatment		Response criteria	Genotypes (n)					
			Dose/day	Follow-up (months)		Responsive			Resistant		
						CC	CA	AA	CC	CA	AA
Takahashi, <i>et al.</i> , 2010	Japan	CP	400 mg (N = 31); 300 mg (N = 17); < 300 mg (N = 14)	NA	MMR	17	11		24	10	
Anthony <i>et al.</i> , 2012	Malaysia	AP, CP	400 mg (N = 45)	≥ 6	Response	1	14	7	10	11	2
Seong <i>et al.</i> , 2013	Korea	CP	400 mg (N = 81)	≥ 6	MMR	5	4	4	36	28	4
Shinohara <i>et al.</i> , 2013	Japan	CP	≤ 400 mg (N = 143); 600 mg (N = 1)	69.6 (median)	CMR	36	28	8	49	20	3
Au <i>et al.</i> , 2014	Malaysia	CP	400 mg (N = 215)	≥ 6	MMR	44	57	17	55	35	7
de Lima <i>et al.</i> , 2014	Brazil	CP	400 mg (N = 118)	≥ 6	MMR	58	11		41	8	
Salimzand <i>et al.</i> , 2015	Iran	AP (N = 11); CP (N = 59)	400 mg (N = 56); 600 mg (N = 14)	64	CR	33	4	12	11	1	9

CML, chronic myeloid leukemia; AP, accelerated phase; CP, chronic phase; CMR, complete molecular response; MMR, major molecular response; CR, cytogenetic response; Response, cytogenetic, molecular, and hematological responses

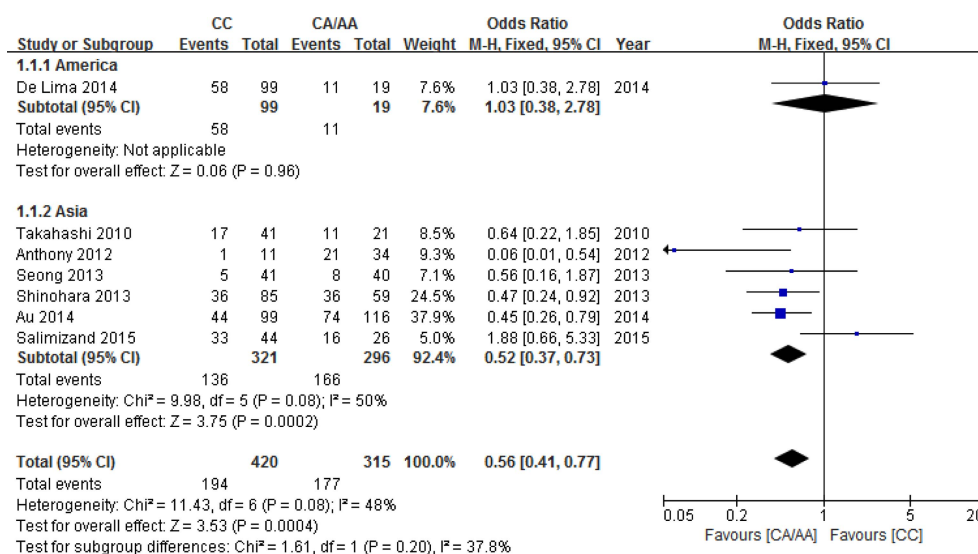


Fig. 2. Forest plot of the association between ABCG2 C421A polymorphism and imatinib response stratified by country under dominant model. Squares represent study-specific OR; horizontal lines represent 95% CIs; diamond represents a summary odds ratio estimate with corresponding 95% CI.

were included for this meta-analysis and 84% of them were Asian. Almost all patients (94%) were treated with 400 mg/day imatinib and more than 92% of the patients were in chronic phase CML. Of the 7 studies, 5 studies show molecular response, 1 study presents cytogenetic response with no degree of the response, and the last 1 reports 'Response' which includes cytogenetic, molecular, and hematologic response. All studies identified the response criteria based on the European LeukemiaNet recommendations: major molecular response, 3 log reduction of BCR/ABL fusion gene transcripts compared with the base line; complete molecular response, 4.5-5 log reduction of BCR/ABL fusion gene transcripts; cytogenetic response categorized as complete (0% Ph+ bone marrow metaphases), partial (1-35% Ph+ metaphases), minor (35-65% Ph+ metaphases), and minimal (65-95% Ph+ metaphases).³⁾ In 5 studies, genotype frequencies were tested for Hardy-Weinberg equilibrium.²²⁻²⁶⁾

The pooled analysis showed that ABCG2 c.421CC genotype was significantly associated with poor response to imatinib under the dominant model (CC vs CA+AA; OR: 0.56; 95% CI: 0.41, 0.77; $p = 0.0004$). The heterogeneity among the studies was not significant ($I^2 = 48\%$; $p = 0.08$). The Asian studies demonstrated a significantly lower response in c.421CC genotype than in c.421CA or c.421AA genotype (OR: 0.52; 95% CI: 0.37, 0.73; $p = 0.0002$) (Fig. 2). Visual inspection of a funnel plot suggested possible publication bias (Fig. 3).

The impact of c.421CC genotype on imatinib response was

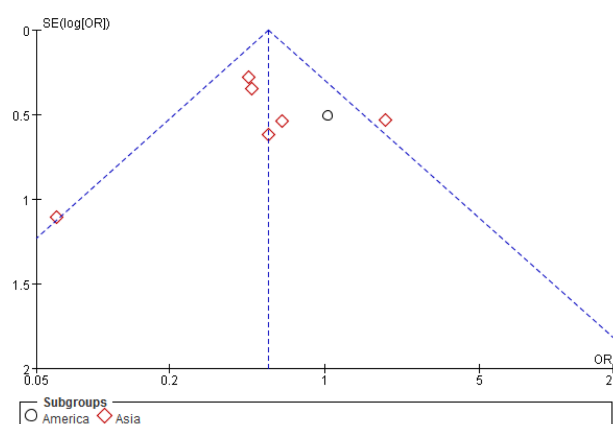


Fig. 3. Funnel plot for publication bias of the included studies in the meta-analysis of the association between ABCG2 C421A polymorphism and imatinib response stratified by country.

also evaluated by analyses of 5 studies reporting the individual frequency of the c.421CC, c.421CA, and c.421AA genotypes. In Asian patients, the c.421CC genotype exhibited higher risk for worse response to imatinib than the c.421CA genotype (heterozygote codominant model: CC vs. AC; OR: 0.49, 95% CI: 0.33, 0.73; $p = 0.0006$). No significant heterogeneity was detected among the studies ($I^2 = 0\%$, $p = 0.44$). When compared with the patients carrying the c.421AA genotype, the subjects with c.421CC had higher risk for poor response to imatinib (homozygote codominant model: CC vs AA; OR: 0.43; 95% CI: 0.25, 0.75, $p = 0.003$). There was a high degree of heterogeneity among the studies ($I^2 = 74\%$; $p = 0.004$) (Fig. 4).

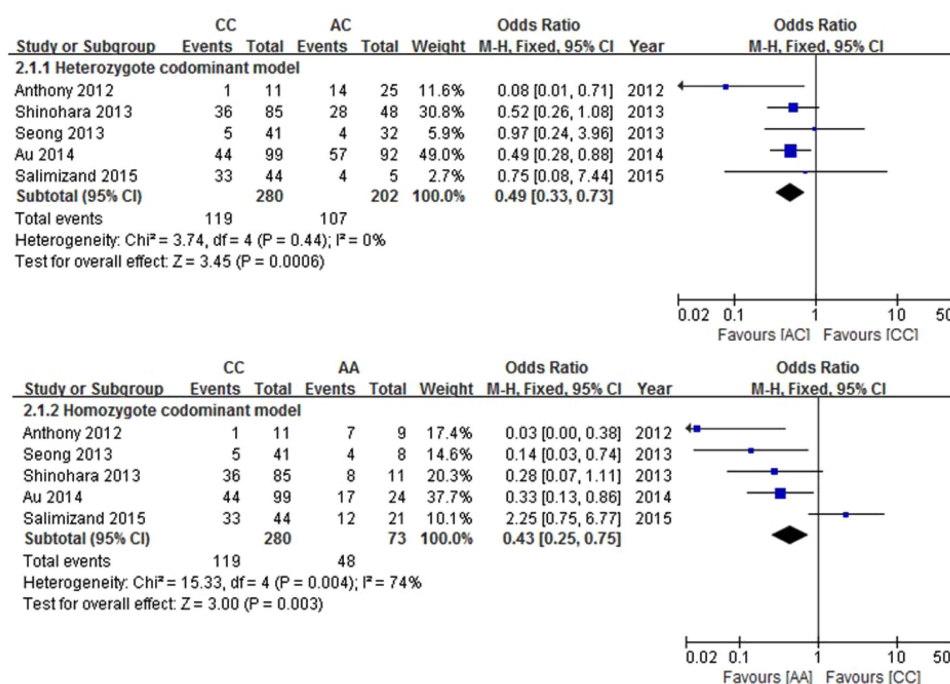


Fig. 4. Forest plot of the association between ABCG2 C421A polymorphism and imatinib response under heterozygote and homozygote codominant model. Squares represent study-specific odds ratio estimates; horizontal lines represent 95% CIs; diamond represents a summary odds ratio estimate with corresponding 95% CI.

Discussion

In the present meta-analysis, ABCG2 c.421CC genotype was significantly associated with poor response to imatinib therapy in CML patients compared to the c.421CA and c.421AA genotypes. Although, the most prominent mechanism of imatinib resistance is mutations or amplification of the BCR-ABL gene, the increase in expression of efflux pumps, such as P-glycoprotein and ABCG2, and the decrease in uptake transporters, including organic cation transporter 1 and organic anion transporting polypeptide 1A2, are also responsible for loss of imatinib efficacy in CML.^{27,28)} ABCG2 is one of the most important efflux transporters associated with imatinib resistance in CML and the C421A polymorphism is most widely studied.

The finding of the present meta-analysis is consistent with the result from a recent study by Skoglund *et al.* In the study, the C421A variants in ABCG2 reduced the expression of ABCG2 protein and increased efficacy of imatinib in CML cells.¹⁵⁾ Takahashi and his colleague also found that imatinib trough concentration of patients with the ABCG2 c.421CC genotype was significantly lower than that of the patients with ABCG2 c.421CA or ABCG2 c.421AA genotype, supporting the result of this meta-analysis.²²⁾ The result of this meta-analysis is also in agreement with a study of gastrointestinal

stromal tumor patients. The study showed that progression-free survival rate in patients with the ABCG2 c.421AA genotype was significantly superior to that of patients with c.421CC or c.421CA genotype.²⁹⁾

The present meta-analysis has pooled the data from 6 studies conducted in Asian countries and 1 study conducted in Brazil. Although the association between the ABCG2 c.421CC genotype and higher risk for imatinib resistance was observed in Asian patients and the Brazilian patients did not exhibit the association, it is difficult to suggest that the association may not be found in non-Asian population.

There are several limitations to this meta-analysis. In the present study, different outcome measures such as CR, CMR, and MMR were used. Another limitation of this study is the lack of information in non-Asian groups. Lastly, the present study was performed in a relatively small number of studies and small sample size. Nevertheless, when comparing the number of studies included in other meta-analyses, it doesn't look like the number of the studies included in the present meta-analysis is too small to evaluate the effect of ABCG2 C421A polymorphism in Asian patients.³⁰⁾

In spite of the limitations, this study does provide information in predicting response to imatinib in Asian CML patients with ABCG2 c.421CC genotype.

Conclusion

The ABCG2 c.421CC genotype was significantly associated with poor response to imatinib therapy in CML compared to the c.421CA and c.421AA genotype, especially in Asian patients.

Conflicts of interest

The authors have no conflict of interest to declare.

References

- Groffien J, Stephenson JR, Heisterkamp N, *et al.* Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. *Cell* 1984;36:93-9.
- Ren R. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. *Nat Rev Cancer* 2005;5:172-83.
- Baccarani M, Deininger MW, Rosti G, *et al.* European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. *Blood* 2013;122:872-84.
- National Institute for Health and Care Excellence. Guidance on the use of imatinib for chronic myeloid leukaemia. Available from <https://www.nice.org.uk/guidance/ta70/chapter/1-guidance>. Accessed January 25, 2016.
- Schindler T, Bornmann W, Pellicena P, *et al.* Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* 2000;289:1938-42.
- Kantarjian HM, Talpaz M, Giles F, *et al.* New insights into the pathophysiology of chronic myeloid leukemia and imatinib resistance. *Ann Intern Med* 2006;145:913-23.
- Burger H, van Tol H, Boersma AW, *et al.* Imatinib mesylate (STI571) is a substrate for the breast cancer resistance protein (BCRP)/ABCG2 drug pump. *Blood* 2004;104:2940-42.
- Brendel C, Scharenberg C, Dohse M, *et al.* Imatinib mesylate and nilotinib (AMN107) exhibit high-affinity interaction with ABCG2 on primitive hematopoietic stem cells. *Leukemia* 2007;21:1267-75.
- Shukla S, Sauna ZE, Ambudkar SV. Evidence for the interaction of imatinib at the transport-substrate site(s) of the multidrug-resistance-linked ABC drug transporters ABCB1 (P-glycoprotein) and ABCG2. *Leukemia* 2008;22:445-7.
- Kondo C, Suzuki H, Itoda M, *et al.* Functional analysis of SNPs variants of BCRP/ABCG2. *Pharm Res* 2004;21:1895-903.
- Stacy AE, Jansson PJ, Richardson DR. Molecular pharmacology of ABCG2 and its role in chemoresistance. *Mol Pharmacol* 2013;84:655-69.
- Kawabata S, Oka M, Soda H, *et al.* Expression and functional analyses of breast cancer resistance protein in lung cancer. *Clin Cancer Res* 2003;9:3052-7.
- Jordanides NE, Jorgensen HG, Holyoake TL, *et al.* Functional ABCG2 is overexpressed on primary CML CD34+ cells and is inhibited by imatinib mesylate. *Blood* 2006;108:1370-3.
- Shah NP. Medical management of CML. *Hematology Am Soc Hematol Educ Program* 2007:371-5.
- Skoglund K, Boiso Moreno S, Jönsson JI, *et al.* Single-nucleotide polymorphisms of ABCG2 increase the efficacy of tyrosine kinase inhibitors in the K562 chronic myeloid leukemia cell line. *Pharmacogenet Genomics* 2014;24:52-61.
- Nambu T, Hamada A, Nakashima R, *et al.* Association of SLCO1B3 polymorphism with intracellular accumulation of imatinib in leukocytes in patients with chronic myeloid leukemia. *Biol Pharm Bull* 2011;34:114-9.
- Yamakawa Y, Hamada A, Nakashima R, *et al.* Association of genetic polymorphisms in the influx transporter SLCO1B3 and the efflux transporter ABCB1 with imatinib pharmacokinetics in patients with chronic myeloid leukemia. *Ther Drug Monit* 2011;33:244-50.
- Wells GA, Shea B, O'Connell D, *et al.* The Newcastle-Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analysis. Ottawa Hospital Research Institute. Available from http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed January 20, 2016.
- Zheng JS, Hu XJ, Zhao YM, *et al.* Intake of fish and marine n-3 polyunsaturated fatty acids and risk of breast cancer: meta-analysis of data from 21 independent prospective cohort studies. *BMJ* 2013;346:f3706.
- Anthony A, Ankathil R, Ai-Sim G, *et al.* Influence of ABCB1 C3435T and ABCG2 C421A Gene Polymorphisms in Response to Imatinib Mesylate in Chronic Myeloid Leukemia Patients. *International Journal of Environmental Science and Development* 2012;3:274-8.
- Shinohara Y, Takahashi N, Nishiwaki K, *et al.* A multicenter clinical study evaluating the confirmed complete molecular response rate in imatinib-treated patients with chronic phase chronic myeloid leukemia by using the international scale of real-time quantitative polymerase chain reaction. *Haematologica* 2013;98:1407-13.
- Takahashi N, Miura M, Scott SA, *et al.* Influence of CYP3A5 and drug transporter polymorphisms on imatinib trough concentration and clinical response among patients with chronic phase chronic myeloid leukemia. *J Hum Genet* 2010;55:731-7.
- Seong SJ, Lim M, Sohn SK, *et al.* Influence of enzyme and transporter polymorphisms on trough imatinib concentration and clinical response in chronic myeloid leukemia patients. *Ann Oncol* 2013;24:756-60.
- Au A, Aziz Baba A, Goh AS, *et al.* Association of genotypes and haplotypes of multi-drug transporter genes ABCB1 and ABCG2 with clinical response to imatinib mesylate in chronic myeloid leukemia patients. *Biomed Pharmacother* 2014;68:343-9.
- de Lima LT, Vivona D, Bueno CT, *et al.* Reduced ABCG2 and increased SLC22A1 mRNA expression are associated with imatinib response in chronic myeloid leukemia. *Med Oncol* 2014;31:851.
- Salimizand H, Amini S, Abdi M, *et al.* Concurrent effects of ABCB1 C3435T, ABCG2 C421A, and XRCC1 Arg194Trp genetic polymorphisms with risk of cancer, clinical output, and response to treatment with imatinib mesylate in patients with chronic myeloid leukemia. *Tumour Biol* 2015; DOI 10.1007/s13277-015-3874-4
- Bixby D, Talpaz M. Mechanisms of resistance to tyrosine kinase inhibitors in chronic myeloid leukemia and recent therapeutic strategies to overcome resistance. *Hematology Am Soc Hematol Educ Program* 2009:461-7.
- Eechoute K, Sparreboom A, Burger H, *et al.* Drug transporters and imatinib treatment: implications for clinical practice. *Clin Cancer Res* 2011;17:406-15.
- Koo DH, Ryu MH, Ryoo BY, *et al.* Association of ABCG2 polymorphism with clinical efficacy of imatinib in patients with gastrointestinal stromal tumor. *Cancer Chemother Pharmacol* 2015;75:173-82.
- Zu B, Li Y, Wang X, *et al.* MDR1 gene polymorphisms and imatinib response in chronic myeloid leukemia: a meta-analysis. *Pharmacogenomics* 2014;15:667-77.