Table 4: Genotyping of ERCC1 C118T polymorphism

	ERCC1 codon 118 genotyping				Allele frequency		*P*
	CC (%)	CT (%)	TT (%)	Total	C	T	
CML cases	51 (29.31)	94 (54.02)	29 (16.66)	174	0.563	0.436	0.467
Controls	49 (28.16)	87 (50.0)	38 (21.83)	174	0.531	0.468	
Gender							
Males	32 (29.90)	57 (53.25)	18 (16.82)	107	0.565	0.434	0.965
Females	19 (28.35)	37 (55.22)	11 (16.41)	67	0.559	0.440	
Age at onset (years)							
<30	14 (26.41)	30 (56.60)	9 (16.98)	53	0.547	0.452	0.852
>30	37 (30.57)	64 (52.89)	20 (16.52)	121	0.570	0.429	
Phase							
Chronic	43 (30.71)	75 (53.57)	22 (15.71)	140	0.575	0.425	0.637
Acute	8 (23.52)	19 (55.88)	7 (20.58)	34	0.514	0.485	
Sokal risk							
Low + intermediate	29 (29.29)	53 (53.53)	17 (17.17)	99	0.560	0.439	0.975
High	22 (29.33)	41 (54.66)	12 (16.0)	75	0.566	0.433	
Hasford risk							
Low + intermediate	36 (27.69)	71 (54.61)	23 (17.69)	130	0.55	0.45	0.663
High	15 (34.09)	23 (52.27)	6 (13.63)	44	0.602	0.397	
EUTOS risk							
Low	34 (29.05)	61 (52.13)	22 (18.80)	117	0.551	0.448	0.543
High	17 (29.82)	33 (57.89)	7 (12.28)	57	0.587	0.412	
BCR-ABL1 levels							
<0.1%	14 (38.88)	17 (47.22)	5 (13.88)	36	0.625	0.375	0.030*
0.1%-10%	17 (25.37)	32 (47.76)	18 (26.86)	67	0.492	0.507	
>10%	20 (28.16)	45 (63.38)	6 (8.45)	71	0.598	0.401	
TKD mutations							
Presence	5 (14.70)	26 (76.47)	3 (8.82)	34	0.529	0.470	0.013*
Absence	46 (32.85)	68 (48.57)	26 (18.57)	140	0.571	0.428	
Present status							
IM 400 mg	22 (35.48)	25 (40.32)	15 (24.19)	62	0.556	0.443	0.143
IM 600 mg and 800 mg	19 (27.94)	39 (57.35)	10 (14.70)	68	0.566	0.433	
Other medicines	4 (25.0)	10 (62.5)	2 (12.5)	16	0.562	0.437	
Died	6 (21.42)	20 (71.42)	2 (7.14)	28	0.571	0.428	

*Significant. EUTOS: European Treatment Outcome Study, TKD: Tyrosine kinase domain, IM: Imatinib, ERCC1: Excision repair cross-complementing protein 1

Arginine genotype and allele frequencies were elevated in patients without TKD mutations. This is the first study to show a correlation between TKD mutations with 72 codon polymorphism. Proline allele increased in TKD mutation carriers and also associated with poor survival in our study. In supporting our data, earlier studies showed association with proline genotype and poor survival.[35,36] The present study indicates that proline genotype with less apoptotic potential efficiency might not be eliminating the cells with DNA lesions and further leading to drug resistance and poor survival in CML patients.

The ERCC1 C118T is a silent polymorphism with varied gene expression levels can alter the DNA repair capacity.[37] Very few studies are available in the literature that correlating ERCC1 118 codon polymorphism with CML.

Heterozygous CT genotype and T allele were found to be significantly increased in patients with high BCR-ABL1 expression levels ($P = 0.030$) and patients carrying with TKD mutations ($P = 0.013$), which indicates that T allele with less repair capability might be associated with resistance to IM treatment in the present study. On contrary Kong *et al.* reported that patients with TT genotype showed association with major and complete cytogenetic response to IM therapy in CML patients.[38] In support to our data, previous studies also showed that patients with CT and TT genotypes were associated with significantly lower response rates in metastatic colorectal carcinoma patients and nonsmall cell lung cancer with platinum-based chemotherapy.[39,40]

Shahnam *et al*. also reported that there was a trend with T allele association with worse outcome in the Asian population.[41] Lu *et al*. observed opposite trend with T allele with better outcome in the Caucasian population.[42] In our study, heterozygous CT genotype associated with poor survival. Heterozygous CT genotype with less DNA repair efficiency might lead to drug resistance and poor survival in CML patients.

Table 5: Distribution of genotype combinations of ERCC1 C118T polymorphism

Model	Genotypes	OR (95% CI)	*P*
Cases versus controls			
Codominant	CC versus CT	1.038 (0.638-1.692)	0.901
	CC versus TT	0.706 (0.401-1.242)	0.253
Dominant	CC versus CT + TT	0.945 (0.594-1.504)	0.905
Recessive	CC + CT versus TT	0.715 (0.418-1.224)	0.276
Over dominant	CC + TT versus CT	1.175 (0.771-1.79)	0.519
Presence versus absence of TKD mutations			
Codominant	CC versus CT	3.517 (1.258-9.830)	0.018*
	CC versus TT	1.061 (0.234-4.805)	1
Dominant	CC versus CT + TT	2.838 (1.031-7.812)	0.05
Recessive	CC + CT versus TT	0.424 (0.120-1.494)	0.207
Over dominant	CC + TT versus CT	3.441 (1.457-8.123)	0.003
<10% versus >10% BCR-ABL1 levels			
Codominant	CC versus CT	0.702 (0.351-1.404)	0.382
	CC versus TT	2.473 (0.856-7.137)	0.135
Dominant	CC versus CT + TT	0.910 (0.467-1.774)	0.865
Recessive	CC + CT versus TT	3.114 (1.197-8.103)	0.021*
Over dominant	CC + TT versus CT	0.524 (0.282-0.973)	0.045*

*Significant. OR: Odds ratio, TKD: Tyrosine kinase domain, CI: Confidence interval

There are no previous Indian studies between BIM deletion polymorphism and CML. We did not find BIM deletion polymorphism in the present study, as India is one of the South Asian countries, this polymorphism might be absent in our study. BIM deletion polymorphism is restricted to East Asian Ancestry (12.3% carrier frequency), but not in the Africans and Europeans.[26]

Conclusion

Our data suggested that proline genotype/allele of 72 codon polymorphism in TP53 gene possibly leading to inefficient apoptosis and 118CT genotype in ERCC1 gene with possibly less DNA repair efficiency might be responsible for suboptimal responses to IM and poor survival in CML patients.

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Conflicts of interest

There are no conflicts of interest.

References

1. Quintás-Cardama A, Kantarjian HM, Cortes JE. Mechanisms of primary and secondary resistance to imatinib in chronic myeloid leukemia. Cancer Control 2009;16:122-31.
2. Slupianek A, Hoser G, Majsterek I, Bronisz A, Malecki M, Blasiak J, *et al.* Fusion tyrosine kinases induce drug resistance by stimulation of homology-dependent recombination repair, prolongation of G(2)/M phase, and protection from apoptosis. Mol Cell Biol 2002;22:4189-201.
3. Koptyra M, Falinski R, Nowicki MO, Stoklosa T, Majsterek I, Nieborowska-Skorska M, *et al.* BCR/ABL kinase induces self-mutagenesis via reactive oxygen species to encode imatinib resistance. Blood 2006;108:319-27.
4. Dulić V, Kaufmann WK, Wilson SJ, Tlsty TD, Lees E, Harper JW, *et al.* P53-dependent inhibition of cyclin-dependent kinase activities in human fibroblasts during radiation-induced G1 arrest. Cell 1994;76:1013-23.
5. Woods DB, Vousden KH. Regulation of p53 function. Exp Cell Res 2001;264:56-66.
6. Pietsch EC, Humbey O, Murphy ME. Polymorphisms in the p53 pathway. Oncogene 2006;25:1602-11.
7. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: Clues to cancer etiology and molecular pathogenesis. Cancer Res 1994;54:4855-78.
8. Thomas M, Kalita A, Labrecque S, Pim D, Banks L, Matlashewski G, *et al.* Two polymorphic variants of wild-type p53 differ biochemically and biologically. Mol Cell Biol 1999;19:1092-100.
9. Bergamaschi G, Merante S, Orlandi E, Galli A, Bernasconi P, Cazzola M, *et al.* TP53 codon 72 polymorphism in patients with chronic myeloid leukemia. Haematologica 2004;89:868-9.
10. Ezzikouri S, El Feydi AE, Chafik A, Benazzouz M, El Kihal L, Afifi R, *et al.* The pro variant of the p53 codon 72 polymorphism is associated with hepatocellular carcinoma in Moroccan population. Hepatol Res 2007;37:748-54.
11. Hadhri-Guiga B, Toumi N, Khabir A, Sellami-Boudawara T, Ghorbel A, Daoud J, *et al.* Proline homozygosity in codon 72 of TP53 is a factor of susceptibility to nasopharyngeal carcinoma in Tunisia. Cancer Genet Cytogenet 2007;178:89-93.
12. Yang W, Zhang Y, Tian X, Ning T, Ke Y. P53 codon 72 polymorphism and the risk of esophageal squamous cell carcinoma. Mol Carcinog 2008;47:100-4.
13. van Duin M, Koken MH, van den Tol J, ten Dijke P, Odijk H, Westerveld A, *et al.* Genomic characterization of the human DNA excision repair gene ERCC-1. Nucleic Acids Res 1987;15:9195-213.
14. Yu JJ, Lee KB, Mu C, Li Q, Abernathy TV, Bostick-Bruton F, *et al.* Comparison of two human ovarian carcinoma cell lines (A2780/CP70 and MCAS) that are equally resistant to platinum, but differ at codon 118 of the ERCC1 gene. Int J Oncol 2000;16:555-60.
15. Furuta T, Ueda T, Aune G, Sarasin A, Kraemer KH, Pommier Y, *et al.* Transcription-coupled nucleotide excision repair as a determinant of cisplatin sensitivity of human cells. Cancer Res 2002;62:4899-902.
16. Reed E. ERCC1 and clinical resistance to platinum-based therapy. Clin Cancer Res 2005;11:6100-2.
17. Kelland L. The resurgence of platinum-based cancer chemotherapy. Nat Rev Cancer 2007;7:573-84.
18. Martin LP, Hamilton TC, Schilder RJ. Platinum resistance: The role of DNA repair pathways. Clin Cancer Res 2008;14:1291-5.
19. Vilmar A, Sørensen JB. Excision repair cross-complementation group 1 (ERCC1) in platinum-based treatment of non-small cell lung cancer with special emphasis on carboplatin: A review of current literature. Lung Cancer 2009;64:131-9.
20. Yu JJ, Mu C, Lee KB, Okamoto A, Reed EL, Bostick-Bruton F, *et al.* A nucleotide polymorphism in ERCC1 in human ovarian cancer cell lines and tumor tissues. Mutat Res 1997;382:13-20.
21. Youle RJ, Strasser A. The BCL-2 protein family: Opposing activities that mediate cell death. Nat Rev Mol Cell Biol 2008;9:47-59.
22. Westphal D, Dewson G, Czabotar PE, Kluck RM. Molecular biology of bax and bak activation and action. Biochim Biophys Acta 2011;1813:521-31.
23. Amarante-Mendes GP, Naekyung Kim C, Liu L, Huang Y, Perkins CL, Green DR, *et al.* Bcr-abl exerts its antiapoptotic effect against diverse apoptotic stimuli through blockage of mitochondrial release of cytochrome C and activation of caspase-3. Blood 1998;91:1700-5.

24. Dubrez L, Eymin B, Sordet O, Droin N, Turhan AG, Solary E, *et al.* BCR-ABL delays apoptosis upstream of procaspase-3 activation. Blood 1998;91:2415-22.
25. Kuroda J, Puthalakath H, Cragg MS, Kelly PN, Bouillet P, Huang DC, *et al.* Bim and bad mediate imatinib-induced killing of bcr/Abl+leukemic cells, and resistance due to their loss is overcome by a BH3 mimetic. Proc Natl Acad Sci U S A 2006;103:14907-12.
26. Ng KP, Hillmer AM, Chuah CT, Juan WC, Ko TK, Teo AS, *et al.* A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nat Med 2012;18:521-8.
27. Augis V, Airiau K, Josselin M, Turcq B, Mahon FX, Belloc F, *et al.* A single nucleotide polymorphism in cBIM is associated with a slower achievement of major molecular response in chronic myeloid leukaemia treated with imatinib. PLoS One 2013;8:e78582.
28. Zhao M, Zhang Y, Cai W, Li J, Zhou F, Cheng N, *et al.* The bim deletion polymorphism clinical profile and its relation with tyrosine kinase inhibitor resistance in Chinese patients with non-small cell lung cancer. Cancer 2014;120:2299-307.
29. Isobe K, Hata Y, Tochigi N, Kaburaki K, Kobayashi H, Makino T, *et al.* Clinical significance of BIM deletion polymorphism in non-small-cell lung cancer with epidermal growth factor receptor mutation. J Thorac Oncol 2014;9:483-7.
30. Chen WH, Xin PL, Pan QX, Chen YY, Wang CR, Zhang ZS, *et al.* ERCC1 single nucleotide polymorphism C8092A, but not its expression is associated with survival of esophageal squamous cell carcinoma patients from Fujian province, China. PLoS One 2014;9:e106600.
31. Zhong J, Li ZX, Zhao J, Duan JC, Bai H, An TT, *et al.* Analysis of BIM (BCL-2 like 11 gene) deletion polymorphism in Chinese non-small cell lung cancer patients. Thorac Cancer 2014;5:509-16.
32. Camelo-Santos J, do Prado Barbosa A, de Paula Silveira-Lacerda E, Guillo LA. Arginine homozygosity in codon 72 of p53 correlates with failure to imatinib response in chronic myeloid leukemia. Biomed Pharmacother 2013;67:103-7.
33. Sailaja K, Surekha D, Nageswara Rao D, Raghunadha Rao D, Balakrishna B, Vishnupriya S. TP53 codon 72 polymorphism and risk of chronic myeloid leukemia. Gene Ther Mol Biol 2009;13:316-20.
34. Weich N, Ferri C, Moiraghi B, Bengió R, Giere I, Pavlovsky C, *et al.* TP53 codon 72 polymorphism predicts chronic myeloid leukemia susceptibility and treatment outcome. Blood Cells Mol Dis 2016;59:129-33.
35. Tommiska J, Eerola H, Heinonen M, Salonen L, Kaare M, Tallila J, *et al.* Breast cancer patients with p53 pro72 homozygous genotype have a poorer survival. Clin Cancer Res 2005;11:5098-103.
36. Schmidt MK, Tommiska J, Broeks A, van Leeuwen FE, Van't Veer LJ, Pharoah PD, *et al.* Combined effects of single nucleotide polymorphisms TP53 R72P and MDM2 SNP309, and p53 expression on survival of breast cancer patients. Breast Cancer Res 2009;11:R89.
37. Komar AA. Genetics. SNPs, silent but not invisible. Science 2007;315:466-7.
38. Kong JH, Mun YC, Kim S, Choi HS, Kim YK, Kim HJ, *et al.* Polymorphisms of ERCC1 genotype associated with response to imatinib therapy in chronic phase chronic myeloid leukemia. Int J Hematol 2012;96:327-33.
39. Chang PM, Tzeng CH, Chen PM, Lin JK, Lin TC, Chen WS, *et al.* ERCC1 codon 118 C→T polymorphism associated with ERCC1 expression and outcome of FOLFOX-4 treatment in Asian patients with metastatic colorectal carcinoma. Cancer Sci 2009;100:278-83.
40. Cheng J, Ha M, Wang Y, Sun J, Chen J, Wang Y, *et al.* A C118T polymorphism of ERCC1 and response to cisplatin chemotherapy in patients with late-stage non-small cell lung cancer. J Cancer Res Clin Oncol 2012;138:231-8.
41. Shahnam A, Ridha Z, Wiese MD, Kichenadasse G, Sorich MJ. Pharmacogenetic and ethnicity influence on oxaliplatin therapy for colorectal cancer: A meta-analysis. Pharmacogenomics 2016;17:1725-32.
42. Lu X, Xiao S, Jin C, van der Straaten T, Li X. ERCC1 and XPD/ERCC2 polymorphisms' predictive value of oxaliplatin-based chemotherapies in advanced colorectal cancer has an ethnic discrepancy: A meta-analysis. J Clin Lab Anal 2012;26:10-5.