

Ultra-deep sequencing mutation analysis of BCR/ABL1 kinase domain in newly diagnosed CML patients

서울대학교병원 내과¹, 충남대학교병원 내과², 울산대학교 아산병원 내과³, 가천대학교병원 내과⁴, 경북대학교병원 내과⁵,
서울대학교 분당병원 내과⁶, 연세대학교 세브란스병원 내과⁷, 순천향대학교병원 내과⁸, 울산대학교병원 내과⁹,
중앙보훈병원 내과¹⁰, 계명대학교 동산병원 내과¹¹, 조선대학교병원 내과¹², 순천향대학교 부천병원 내과¹³,
대구 가톨릭대학교병원 내과¹⁴, 강동 성심병원 내과¹⁵, 분당 차병원 내과¹⁶, 삼성병원 내과¹⁷, 인제대학교 해운대 백병원 내과¹⁸

오재익¹, 김민호¹, 김형준², 신동엽¹, 김대영³, 이규형³, 안재숙², 박진희⁴, 손상곤⁵, 이윤진⁵, 이정옥⁶, 정준원⁷,
김경하⁸, 김혁⁹, 남승현^{1,10}, 도영록^{1,11}, 박상곤^{1,2,12}, 박성규^{1,3,13}, 배성화^{1,4,14}, 송현호^{1,5,15}, 오도연^{1,6,16},
정철원^{1,7,17}, 박선양^{1,8,18}, 박현경¹

Background/Aims: Ultra-deep sequencing detects low frequency genetic mutations with high sensitivity.

Methods: we used ultra-deep sequencing to prospectively examine mutations in the BCR/ABL1 tyrosine kinase domain of newly diagnosed, chronic-phase chronic myeloid leukemia (CML) patients treated with the tyrosine kinase inhibitor, nilotinib.

Results: Between May 2013 to November 2014, 50 patients from 18 institutions were enrolled. We screened 103 somatic mutations and found that mutations in the P-loop domain were the most frequent (173/454 mutations in the P-loop). Additionally, the V299L mutation (dasatinib-resistant/nilotinib-sensitive) was observed in 98% of patients (49/50). None of the patients had Y253H, E255V, or F359V/C/I mutations that were recommend dasatinib rather than nilotinib treatment. Interestingly, the S417Y mutation was associated with lower achievement of major molecular response (MMR) at 6 months, and the V371A mutation was associated with reduced MMR and MR4.5 durations (MMR for 2 years: 100% for no mutation vs. 75% for mutation, P=0.039; MR4.5 for 15 months: 94.1% vs. 25%, P=0.002). Patients who had known nilotinib-resistant mutations demonstrated lower rates of MR4.5 achievement.

Conclusions: In conclusion, ultra-deep sequencing is a sensitive method for genetic-based treatment decisions. Based on these mutational analyses, nilotinib treatment is a promising option for Korean CML patients.

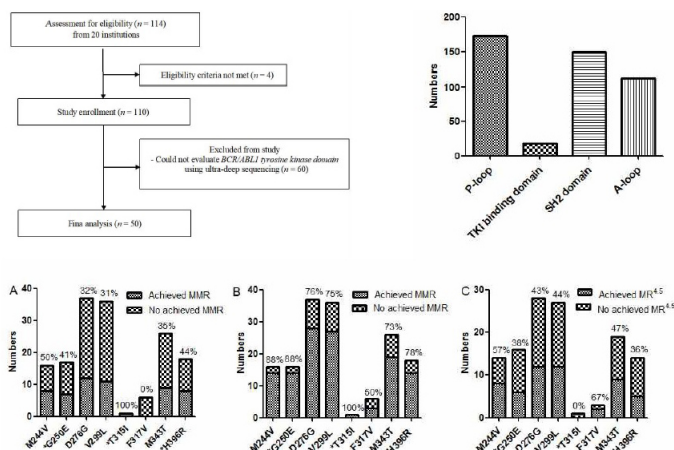


Figure 1. CONSORT flow diagram.

Figure 2. The frequency of mutations according to each domain in BCR/ABL1 tyrosine kinase domain.

Figure 3. The achievement of (A) MMR at 6 months, (B) MMR at 12 months, and (C) MR4.5 at 22 months after the initiation of nilotinib treatment according to the major mutations.

Table 1. List of mutations in the BCR/ABL1 kinase domain.

Mutation	Frequency (%)	Mutation	Frequency (%)
M244V	21.10 (42)	D345V	0.50 (0)
T248V	0.50 (0)	L364I	2.50 (4)
G250R	23.50 (46)	A365V	43.50 (86)
G250R	27.50 (54)	L370P	37.50 (74)
G252H	0.50 (0)	V371A	8.50 (16)
Y253H	0.50 (0)	E373K	3.50 (6)
E255K	1.50 (3)	V379I	0.50 (0)
E255V	0.50 (0)	A380T	0.50 (0)
E258D	0.50 (0)	F382L	0.50 (0)
E273M	0.50 (0)	L384M	0.50 (0)
E279K	0.50 (0)	L387M	0.50 (0)
V280A	0.50 (0)	V393C	43.50 (86)
V280A	0.50 (0)	H396P	0.50 (0)
V299L	49.50 (98)	H396R	25.50 (50)
F311L	1.50 (3)	A397P	0.50 (0)
F311L	1.50 (3)	G399R	13.50 (26)
F317V	8.50 (16)	S417Y	13.50 (26)
F317V	2.50 (5)	S417Y	1.50 (3)
V342H	44.50 (88)	E453K	1.50 (3)
M342T	36.50 (72)	E453K	0.50 (0)
A344V	14.50 (28)	E459V	0.50 (0)
M351T	1.50 (3)	E459K	0.50 (0)
E355D	0.50 (0)	F480L	0.50 (0)
F359V	0.50 (0)	F486S	14.50 (28)

Table 2. Achievement of major molecular response (MMR) at 6 months after initiation of nilotinib treatment.

Variables	Univariate analysis		Multivariate analysis	
	Achievement of MMR at 6 months	P-value	OR	P-value
Age, years	≤53	0.719 (36.8)	1.000	
	>53	0.554 (25.2)		
Gender	Male	0.522 (36.4)	0.728	
	Female	0.554 (25.2)		
Initial WBC	≤46,000/μL	0.516 (31.3)	0.554	
	>46,000/μL	0.717 (44.2)		
Initial Hb	≥12.0 g/dL	0.718 (38.9)	0.480	
	<12.0 g/dL	0.517 (27.9)		
Initial platelet	≥400,000/μL	0.718 (38.9)	0.480	
	<400,000/μL	0.517 (27.9)		
Initial peripheral blast	Present	0.411 (36.4)	1.000	
	Absent	0.516 (31.3)		
Duration from diagnosis to treatment	≤7 days	10.223 (40.0)	0.725	
	>7 days	8.282 (35.0)		
Mutation burden	≤17	9.221 (40.9)	0.307	
	>17	8.282 (35.0)		
P-loop mutations	Present	9.313 (29.0)	0.064	
	Absent	8.282 (35.0)		
S417Y mutation	Present	1.111 (0.9)	0.304	0.733
	Absent	1.111 (0.9)		0.733

Table 3. Achievement of molecular response at 4.5-log reduction (MR4.5) at 22 months after initiation of nilotinib treatment.

Variables	Univariate analysis		Multivariate analysis	
	P-value	OR	P-value	OR
Age, years	≤53	0.614 (33.3)	0.263	
	>53	0.719 (24.4)		
Gender	Male	0.815 (13.0)	0.696	
	Female	0.877 (23.3)		
Initial WBC	≤46,000/μL	0.714 (58.0)	0.671	
	>46,000/μL	0.784 (58.0)		
Initial Hb	≥12.0 g/dL	0.714 (58.0)	0.623	0.295-32.786
	<12.0 g/dL	0.714 (58.0)		
Initial platelet	≥400,000/μL	0.612 (53.0)	0.508	
	<400,000/μL	0.612 (53.0)		
Initial peripheral blast	Present	10.015 (40.0)	0.645	
	Absent	10.015 (40.0)		
Duration from diagnosis to treatment	≤7 days	1.712 (28.0)	0.330	
	>7 days	1.712 (28.0)		
Mutation burden	≤17	1.000	1.000	
	>17	8.19 (42.1)		
P-loop mutations	Present	0.415 (75.0)	0.285	
	Absent	0.415 (75.0)		
L370P mutation	Present	0.057	0.057	8.239
	Absent	0.057		0.655-103.654
V371A mutation	Present	0.053	0.053	
	Absent	0.053		
A380T mutation	Present	0.024	0.024	
	Absent	0.024		0.999

Abbreviations: WBC, white blood cell; Hb, hemoglobin; OR, odds ratio; CI, confidence interval.