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Large-Scale Analysis of *KIT* Aberrations in Chinese Patients with Melanoma

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Abstract

Purpose: *KIT* aberrations were described in acral and mucosal melanomas in largely Caucasian populations. Asian populations are more prone to develop acral and mucosal than cutaneous melanomas, and may harbor a high frequency of *KIT* aberrations.

Experimental Design: Melanoma subtypes ($n = 502$) were analyzed histologically to determine melanoma subtype. Tissue samples were analyzed for mutations in exons 9, 11, 13, 17, and 18 of *KIT* gene in genomic DNA by PCR amplification and Sanger sequencing. The copy numbers of the *KIT* gene were analyzed by quantitative PCR, and protein expression levels of KIT (CD117) were determined by immunohistochemistry.

Results: The most common melanoma subtypes were acral (38.4%) and mucosal (33.3%) melanomas in this population. The overall incidence of somatic mutations within the *KIT* gene was 10.8% (54/502), and all subtypes of melanoma contained *KIT* mutations. Increases in *KIT* gene copy numbers were correlated to CD117 overexpression. The genetic mutations of *KIT* were unrelated to the age, gender, stage, thickness, and ulceration of primary melanomas. Importantly, the overall survival of melanoma patients with *KIT* mutations ($P = 0.001$) or with *KIT* aberrations (mutation plus amplification, $P = 0.0002$) was significantly shorter than that of patients without such alterations.

Conclusion: In China, the prevalent melanomas are acral and mucosal melanomas. *KIT* mutations are detected in all melanoma subtypes. Our study suggests that increases in *KIT* gene copy numbers, but not *KIT* mutations, may be correlated to CD117 overexpression. For the first time, our study suggests that genetic *KIT* aberration is an adverse prognostic factor for melanoma. *Clin Cancer Res; 17(7); 1684–91. ©2011 AACR.*

Introduction

The incidence of melanoma is rising globally and this includes an increased prevalence in China (1–3). Based on the anatomic location and the degree of sun exposure, melanomas can be classified into 4 subtypes: (1) melanomas that occur on skin without chronic sun-induced damage (non-CSD); (2) melanomas on skin with chronic sun-induced damage (CSD); (3) mucosal melanomas, and (4) acral melanomas (4, 5). Incidence of these 4 subtypes differs significantly among different races and ethnicities (1–3). For example, acral melanomas account for only 1% to 7% of all cutaneous melanomas in Caucasians, whereas the percentage is significantly higher in Chinese and other populations (1–3, 6, 7). Despite these apparent disparities, few investigations have rigorously evaluated melanoma subtypes in large non-Caucasian cohorts, and no studies have been performed in Chinese patient populations.

The *KIT* (c-Kit, CD117, stem cell factor receptor) proto-oncogene encodes a 145 kDa transmembrane tyrosine kinase protein (8). The dysregulation of KIT has been reported in certain neoplastic disorders (9, 10). Signal transduction pathways, such as the PI3K/Akt and mitogen-activated protein (MAP) kinase pathways, have been implicated in mediation of KIT-induced mitogenesis and differentiated functions (11). Sequencing of the *KIT* gene has revealed mutations in the majority of gastrointestinal stromal cell tumors (GIST). Recently, Curtin and colleagues, who examined 102 primary melanomas excised from

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Translational Relevance

The incidence of melanoma is rising globally and this includes increased prevalence in China. Asian populations are more prone to develop acral and mucosal than cutaneous melanomas. *KIT*, an important proto-oncogene, has been implicated in the progression of melanoma. As *KIT* aberrations were described in acral and mucosal melanomas in retrospective series of largely Caucasian populations, it was hypothesized that Asian populations will harbor a high frequency of *KIT* aberrations and provide a larger pool of potential clinical trial participants for KIT inhibitors. Preclinical investigations of *KIT* aberrations in large scale of Chinese population may thus benefit the choice of kinase inhibitors for melanoma treatment.

various anatomical sites, found mutations and/or copy number increases of *KIT* in 39% of mucosal melanomas, 36% of acral melanomas, and 28% of melanomas on chronically sun-damaged skin, but not in any (0%) melanomas on nonglabrous skin without CSD (5). Other recent studies also identified oncogenic *KIT* mutations in several melanoma cohorts (4, 12–15). These findings indicate that *KIT* is an important oncogene in melanomas of mucosa, acral skin, and skin with CSD.

Imatinib (formerly known as STI571) is a tyrosine kinase inhibitor with activity against Abelson tyrosine kinase (ABL), KIT, and platelet-derived growth factor receptors (11). This drug is approved for the treatment of chronic myelogenous leukemia and GIST, and is being evaluated in clinical trials for the treatment of melanomas harboring *KIT* mutations (16–22). KIT is a validated therapeutic target in GIST, with a large percentage of patients bearing *KIT* mutant tumors benefiting from imatinib and second-generation KIT inhibitors (16, 17). Prior trials have shown that kinase inhibitors such as imatinib have little or no activity in histologically or genetically unselected melanoma patients (18–20). Therefore, ongoing trials require that a *KIT* gene mutation be documented in the tumor before a patient is treated (21, 22). However, published series on the frequency of *KIT* aberrations in melanoma are relatively small, and there are almost no data available on Chinese patients, with potentially the largest population of such patients.

We collected 502 melanoma tissue samples from patients, evaluated incidence of melanoma subtypes and examined all samples for alterations in the *KIT* gene. This study represents the first systematic analysis of melanoma subtypes and somatic *KIT* mutations of melanoma in a Chinese patient cohort.

Patients and Methods

Patients and tumor tissue samples

This study involved samples from 502 melanoma patients, hospitalized during January 2006 and January 2010 at the Peking Cancer Hospital & Institute. These samples were analyzed by hematoxylin and eosin (H&E) staining and by immunohistochemistry for melanoma markers (S-100, HMB-45, or MART-1) to confirm the diagnosis of melanoma. Sun-induced damage was defined microscopically by the presence or absence of marked solar elastosis on H&E-stained sections, and was determined by at least 2 individual pathologists as previously described (4, 5). Clinical data, including age, sex, TNM (tumor-node-metastases) stage, thickness (Breslow), ulceration, and survival (follow-up persisted until the missing of follow-up or the death of patients) were collected. A representative formalin-fixed, paraffin-embedded block was selected and used for immunohistochemical (IHC) analysis of CD117 as well as for the molecular studies. Samples were sequentially subjected to molecular analysis of *KIT* mutations and *KIT* gene copy numbers (the process for collection and analysis of the samples is illustrated in Supplementary Fig. S1, and detailed information for these 502 cases is listed in Supplementary Table S1). To ensure the accuracy of analysis for *KIT* mutations, results for the first 20 melanoma samples were independently confirmed in Dr. Corless' laboratory, Department of Pathology, Oregon Health and Science University, Portland, USA. This study was approved by the medical ethics committee of the Beijing Cancer Hospital & Institute and was conducted according to the Declaration of Helsinki Principles.

DNA preparation and mutation screening

Genomic DNA was extracted from formalin-fixed, paraffin-embedded sections using a QIAamp DNA FFPE Tissue Kit (Qiagen). To detect hotspot mutations, we amplified exons 9, 11, 13, 17, and 18 of the *KIT* gene by PCR in at least 2 separate preparations of genomic DNA. The primer sequences are listed in Supplementary Table S2. PCR conditions have been described previously (5, 13, 15, 23–25). We purified PCR products with QIAquick (Qiagen), and directly sequenced them using Big Dye Terminator sequencing chemistry on an ABI 3130 automated sequencer (Applied Biosystems). All mutations were confirmed by repeat bidirectional sequencing on the ABI sequencer.

KIT gene amplification analysis by real-time PCR

Quantitative real-time PCR was performed as described previously (15, 23), using ribonuclease P (RNase P) as a control gene. Relative copy numbers were calculated using the $\Delta\Delta C_t$ method (as detailed in Supplementary Table S2).

CD117 immunohistochemistry

IHC analysis for CD117 (KIT protein) was done using the Dako polyclonal rabbit antibody (Dako) at 1:400 dilution, followed by a standard avidin–biotin detection protocol using diaminobenzidine. Hematoxylin–counterstained slides were cover-slipped and examined for the intensity of staining. The staining intensity was scored as 0, 1, 2, and 3 ("0" as negative, and "3" as the strongest) by 3 pathologists independently, and examples of the scores were shown in Supplementary Fig. S2.

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Table 1. Patient characteristics and melanoma subtypes in Chinese patients

Group	Patients		Sex			Age	
	No.	%	Male	Female	M/F (%)	Median	Range
Acral	193	38.4	105	88	119.3	56	22–87
Mucosal	167	33.3	71	96	74.0	53	23–82
CSD	29	5.8	14	15	93.3	55	32–81
Non-CSD	62	12.4	24	38	63.2	52	20–82
UP	51	10.2	26	25	104	56	22–83
Total	502	/	240	262	91.6	54	20–87

Abbreviations: M, male; F, Female.

Statistical analysis

All the statistical analyses were performed using SPSS 13.0 software. Categorical data are described using frequencies and percentages. Continuous data such as age are described using means ± SD for normally distributed data. χ^2 test or Fisher's exact test or Kruskal–Wallis test was used to differentiate the rates of different groups, and differences in measurement data of 2 groups were evaluated by unpaired *t* test or *t*' test. Survival curves were established using the Kaplan–Meier method and compared by the log-rank test. All statistical analyses were 2 sided, and significance was assigned at $P < 0.05$.

Results

Melanoma subtypes in a Chinese population

Using the classification scheme developed by Bastian and colleagues (4, 5), we found that, in our Chinese patient cohort, the most prevalent melanoma subtypes were acral (38.4%) and mucosal (33.3%) melanomas (Table 1). The CSD melanomas were relatively rare (5.8%) in Chinese. Non-CSD melanomas, by far the most common subtype in Caucasians, accounted for 12.4% of all melanomas in Chinese population. Additionally, 51 patients with melanomas (e.g., melanomas found in lymph nodes, liver, lung, and brain upon hospitalization) of unknown primary (UP), accounted for 10.2% of all melanomas.

Genetic aberrations of *KIT* gene in melanoma subtypes

To investigate mutations within *KIT* in Chinese patients, we amplified mutation hotspot regions (exons 9, 11, 13, 17, and 18) of *KIT* gene and analyzed the PCR products by Sanger sequencing. To ensure accurate results, we performed identical experimental processes (DNA extraction, PCR amplification and bidirectional sequencing) at least twice on each sample. Typical sequencing results for mutations in *KIT* are shown in Supplementary Figures. S3 to S8.

Among the 502 samples screened for *KIT* mutations, the overall mutation frequency was 10.8% (54/502), with the highest mutation frequency within the CSD subgroup (20.7%). In the acral and mucosal melanoma subtypes, the frequency of *KIT* mutations was 11.9% (23/193) and 9.6% (16/167), respectively (Table 2), which are lower than the mutation frequency reported in Caucasian patients (5, 13). We also examined *KIT* gene copy number in these samples and found that it was increased in 37 (7.4%) of the 502 samples. Increased *KIT* gene copy number was comparatively more frequent in acral (7.3%) and mucosal (10.2%) melanomas than in CSD (3.4%) and Non-CSD (3.2%) melanomas (Table 2). In the entire cohort of 502 patients, genetic aberrations (mutation plus amplification) of *KIT* were detected in 86 (17.1%) cases (5 of them harboring both *KIT* mutation and increased *KIT* copy number). *KIT* aberrations were detected in 17.6%

Table 2. Genetic aberrations of *KIT* in Chinese melanoma subtypes

Subtype	*KIT* mutation			Increased *KIT* copy number			Genetic *KIT* aberration		
	No.	Positive	%	No.	Positive	%	No.	Positive	%
Acral	193	23	11.9	193	14	7.3	193	34	17.6
Mucosal	167	16	9.6	167	17	10.2	167	32	19.2
CSD	29	6	20.7	29	1	3.4	29	6	20.7
Non-CSD	62	5	8.1	62	2	3.2	62	7	11.3
UP	51	4	7.8	51	3	5.9	51	7	13.7
Total	502	54	10.8	502	37	7.4	502	86	17.1

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Table 3. CD117 expression in melanoma subtypes

Subtype	IHC scores[a]				NES	NPS[b]	%
	0	1	2	3			
Acral	121	57	6	9	193	72	37.3
Mucosal	93	51	12	11	167	74	44.3
CSD	20	8	0	1	29	9	31
Non-CSD	35	18	7	2	62	27	43.5
UP	34	11	4	2	51	17	33.3
Total	303	145	29	25	502	199	39.6

Abbreviations: NES, number of examined samples; NPS; number of positive samples.
[a]The signal intensity of immunohistochemistry results were determined by 3 individual pathologists and scored as 0, 1, 2, and 3, with score "0" as negative and score "3" as the strongest.
[b]Samples with signal intensity of scores 1, 2 or 3 were regarded as CD117 positive.

(34/193) of acral melanomas, 19.2% (32/167) of mucosal melanomas, 20.7% (6/29) of CSD melanomas, 11.3% (7/62) of Non-CSD melanomas, and 13.7% (7/51) of melanomas of UP.

CD117 expression levels in melanoma subtypes

Among the 502 samples, the overall rate of detection of CD117 staining was 39.6% (199/502; Table 3). Expression of CD117 protein was observed in 37.3% (72/193) of acral, 44.3% (74/167) of mucosal, 31.0% (9/29) of CSD, 43.5% (27/62) Non-CSD, and 33.3% (17/51) of melanomas UP (Table 3). We found that the expression of CD117 was not significantly different between these subtypes ($P = 0.40$).

Next, we analyzed the correlation of *KIT* mutations and increased *KIT* gene copy number to CD117 expression levels. Among the 54 cases with *KIT* mutations, the IHC detection rate for CD117 was 44.4% (24/54), which was not significantly higher ($P = 0.55$) than that (39.1%) in cases (175/448) without *KIT* mutations (Table 4). For the 37 cases showing increased *KIT* gene copy number, the detection rate for CD117 was 59.5% (22/37), which was significantly higher ($P = 0.02$) than that (38.1%) in cases (177/465) with normal *KIT* gene copy number (Table 4). However, when the data for *KIT* gene mutation were combined with the data for *KIT* gene amplification (regarded as *KIT* genetic aberrations), we found that the

Table 4. Correlation of *KIT* genetic aberrations to CD117 expression

Subtype	*KIT* mutation		Gene copy number		Genetic aberration[a]	
	Mutation	WT	Increased	Normal	Aberrated	Non
IHC scores[b]						
0	30	273	15	288	43	260
1	16	129	13	132	28	177
2	5	24	5	24	9	20
3	3	22	4	21	6	19
Positive/total	24/54	175/448	22/37	177/465	43/86	156/416
Positive rate (%)	44.4	39.1	59.5	38.1	50	37.5
P[c]	0.55[d]	–	0.02[e]	–	0.04[f]	–

Abbreviations: WT, wild type.
[a]Genetic aberration includes *KIT* mutation and increased gene copy number. Five cases show both genetic mutation and increased gene copy number of *KIT*.
[b]The signal intensity of immunohistochemistry results were determined by 3 individual pathologists and scored as 0, 1, 2, and 3 with score "0" as negative and score "3" as the strongest.
[c]Significance evaluated by chi-square tests.
[d]Positive rate of CD117 in cases with *KIT* mutation versus that in cases without *KIT* mutation.
[e]Positive rate of CD117 in cases with increased *KIT* gene copy number versus that in cases with normal *KIT* gene copy number.
[f]Positive rate of CD117 in cases with *KIT* genetic aberrations versus that in cases without *KIT* aberrations.

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Table 5. *KIT* aberrations in melanoma subtype

Subtype	*KIT* mutation	Domain affected	Incresed *KIT* copy number (no./total)[a]	CD117 expression (no./total)[b]
Acral	V489I; E490G; Y553N; I563V; W582stop; N566D + A829V; L576P; L576F + A636A; E633G; L637L + G648S; K642E; I817T; N822K; C844Y; F848L; W853stop; L859P + L865L	ECD; JMD; kinase; JMD + kinase	3/23	8/23
Mucosal	Q556R; W557R; V559A; V560D; E561G; I571-L576del; L576P; E583G; K642E; L647F; I789T; D816N; N822K; N822Y; W853stop	JMD; kinase	1/16	11/16
CSD	V555I; I571M; K642E; L656R; Y846H; E849stop	JMD; kinase	1/6	1/6
Non-CSD	P551L; I571-L576del; T632I+L637F; K642E; L831P	JMD; kinase	0/5	3/5
UP	L576P; L576F; P577H; S645N	JMD; kinase	0/4	1/4

[a]The threshold for *KIT* gene copy number increase was 3.40 copies of *KIT* relative to RNase P. "No/total" indicates for number of cases with increased *KIT* copy number to that of cases with *KIT* mutation.
[b]The signal intensity of immunohistochemistry results were determined by 3 individual pathologists and scored as 0, 1, 2, and 3. Samples with signal intensity of scores 1, 2, or 3 were regarded as CD117 positive. "No/total" indicates for number of cases positive for CD117 to that of cases with *KIT* mutation.

positivity rate of CD117 IHC (50.0%) in cases (43/86) with genetic *KIT* aberrations was significantly higher ($P = 0.04$) than that (37.5%) in cases (156/416) without genetic *KIT* aberrations. These data indicate that *KIT* mutation may not necessarily lead to increased CD117 expression, but the amplification of *KIT* gene does correlate with CD117 overexpression. Importantly, these results suggest that IHC analysis of CD117 expression is an unreliable surrogate for *KIT* aberration analysis and should not be used as an initial screen to identify *KIT* genetic aberrations.

Alterations of *KIT* in melanoma subtypes

KIT aberrations, including *KIT* gene mutations and increased *KIT* gene copy number, and the expression of KIT protein have been implicated in the pathogenesis of certain human malignancies (9–11). Of the 54 cases with *KIT* mutations, 39 different mutations were detected (Table 5 and Supplementary Fig. S3–S8). In addition to 14 known *KIT* mutations (E490G, P551L, Y553N, W557R, V559A, V560D, N566D, L576P, L576F, W582Stop, K642E, D816N, N822K, and N822Y; refs 4, 5, 9, 11–13, 15), we found 25 novel *KIT* mutations. In our cohort, the most frequent *KIT* mutations were L576P (9 cases), K642E (5 cases), I571-L576 in-frame deletion (2 cases), and N822K (2 cases). Point mutations resulting in single amino acid substitutions (totaling 33 mutations detected in 47 patients) were the most common type of *KIT* mutation. Additionally, there were 2 cases with mutations in 2 separate exons (codes No. 146 and No. 334, Supplementary Fig. S8), 4 cases with premature stop codons (cases No. 245, No. 263, No. 338, and No. 447), and 2 cases with in-frame deletions in exon 11 (IDPTQL, amino acids 571–576, cases No. 26 and No. 501). The 39 different types of *KIT* mutations detected in this study affect the extracellular domain (ECD, 2 cases), the juxtamembrane domain (JMD, 26 cases), the kinase domain (kinase, 25 cases), or both the JMD and the kinase domain (1 case; code No. 146) of KIT protein. These data suggest that the JMD and the kinase domain are affected most frequently, and more effects are required to document the functional significance of novel *KIT* mutations.

Among the 54 cases with a *KIT* mutation, 5 cases (9.3%) showed increased *KIT* gene copy number and 24 cases (44.4%) were positive for CD117 (Table 5), which was not significantly different to those (7.4% and 39.6% respectively) detected in the overall population. Moreover, we found that 68.8% (11/16) of mucosal melanomas with *KIT* mutations were positive for CD117, which, however, was not significantly higher than the staining seen in acral melanomas (34.8%), CSD melanomas (16.7%), Non-CSD melanomas (60.0%), or of melanomas of UP (25.0%; $P = 0.11$). These data suggest that *KIT* mutation is not necessarily related to increased *KIT* gene copy number or CD117 expression.

Correlation of *KIT* aberrations to the clinical features of melanoma

In our cohort, the mean age and the proportion of gender were not significantly different between patients with *KIT* mutation and those without *KIT* mutation

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(Supplementary Table S3). The age and gender proportion were also similar between groups with or without increased *KIT* copy number, *KIT* genetic aberrations (mutation plus amplification) or CD117 positive expression (Supplementary Table S3).

Stage, thickness and ulceration of melanoma lesions are important clinical features of melanoma, and these factors have been implicated in the prognosis of melanoma (1, 26). The data for stage (450 cases), thickness (438 cases) and ulceration (439 cases) were available for further analysis in our study (detailed in Supplementary Table S1).

Among the 51 patients with *KIT* mutations and with the clinical stage data available, the percentages of patients with stage I, II, III, and IV were 3.9% (2 cases), 37.3% (19 cases), 21.6% (11 cases), and 37.3% (19 cases), respectively, which were not significantly different from those without a *KIT* mutation (Supplementary Table S3). For the patients with *KIT* amplification (32 cases), the percentages of the 4 stages were also not different to those in patients showing normal *KIT* gene copy number (Supplementary Table S3). When *KIT* mutation plus *KIT* amplification were regarded as a single factor (*KIT* genetic aberration), the stage distribution was similar between patients with or without *KIT* genetic aberrations. The proportion of cases with overexpression of CD117 versus the absence of expression did not vary across the 4 stages of melanoma (Supplementary Table S3). Moreover, in the patients with advanced stage IV melanoma, data were available for 67 patients with M1a/b melanomas and 79 patients with M1c melanomas, respectively. We found that the genetic mutations, amplifications, and aberrations (mutation plus amplification) of *KIT* were not significantly different between M1a/b and M1c stages ($P = 0.54$, 0.78, and 0.80, respectively).

The average thickness of all 438 available samples was more than 5 mm, which was much thicker than the previous reports (1, 2) but was the actual status for Chinese patients upon hospitalization. The average thickness of samples without *KIT* mutations was 4.91 ± 2.54 mm, whereas that of samples with *KIT* mutations was 5.24 ± 2.63 mm, the tumor thickness was not significantly different in these 2 groups ($P = 0.46$, chi-square test). Moreover, the average thickness in patients with *KIT* gene amplification (5.08 ± 2.60 mm), *KIT* genetic aberrations (5.24 ± 2.55 mm), or CD117 overexpression (4.92 ± 2.61 mm) were not significantly different to those ($P = 0.84$, 0.89 and 0.75, respectively) in patients without such *KIT*/CD117 alterations (Supplementary Table S3).

Ulceration of a cutaneous melanoma on microscopic sections is an adverse prognostic finding (1, 26). In our cohort, the overall ulceration rate was 59.2% (260/439). Specifically, the ulceration rate in acral, mucosal, CSD and Non-CSD melanomas were 60.4% (113/187), 56.2% (91/162), 69.0% (20/29), and 59.0% (36/61), respectively. Moreover, we found that statistical differences were not found for ulceration rates between melanomas with or without *KIT* mutations ($P = 0.65$), with or without *KIT* amplification ($P = 0.46$), with or without genetic *KIT* aberrations ($P = 0.61$) or with or without CD117 overexpression ($P = 0.22$; Supplementary Table S3).

Prognostic significance of *KIT* aberrations for overall survival of melanoma

The stage, thickness, and ulceration of primary melanoma are known prognostic factors for prediction of outcomes of melanoma (1, 26). Although several studies have investigated *KIT* gene mutations in melanoma (4, 5, 12–15), their small size precluded meaningful investigation into the relationship of *KIT* alterations to overall survival. In consistent with previous study (27), we found that the overall survival of patients with ulceration or at advanced stages was significantly shorter than those without ulceration ($P < 0.05$) or at an early stage ($P < 0.001$), respectively. Then we analyzed the prognostic significance of *KIT* mutation, *KIT* amplification, *KIT* genetic aberrations (mutation plus amplification), and CD117 expression for overall survival. The survival data were collected for patients who were diagnosed as primary melanoma or melanoma of UP (Supplementary Table S1). The median follow-up period was 24.00 (3.00 ~ 229.00) months ($n = 473$). We found that the median survival time for patients with *KIT* mutations (30 months) was significantly shorter than for patients with wild-type tumors (53 months; $P = 0.01$, Fig. 1A). In addition, patients with increased *KIT* copy number had a worse survival (42 months) than patients with normal *KIT* copy number (53 months; Fig. 1B). Together, *KIT*

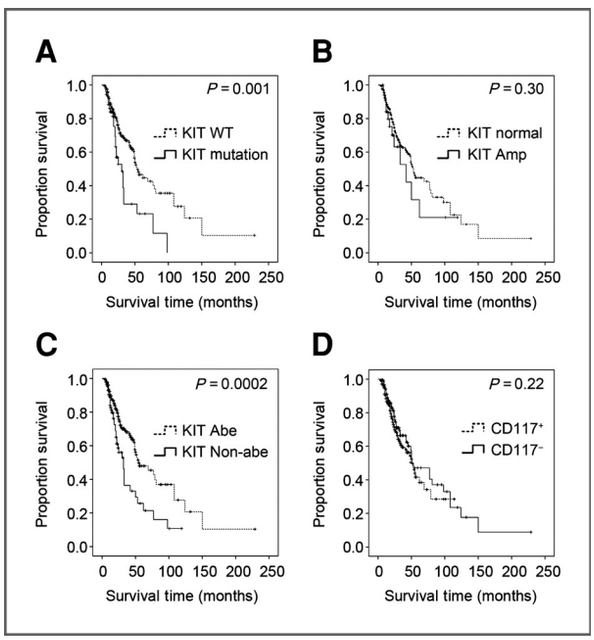

Figure 1. Overall survival of melanoma patients in relation to *KIT* mutations (A), gene copy numbers of *KIT* (B), genetic *KIT* aberrations (C), and CD117 overexpression (D). WT, wild type; Amp, amplification; Abe, aberration; Non-Abe, nonaberration.

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genetic aberrations were also associated with shorter survival (32.00 months vs. 55.00 months; $P = 0.0002$, Fig. 1C), whereas there was no difference in the outcome of cases with CD117 overexpression (52 months) as compared with cases lacking CD117 expression (51 months; Fig. 1D). These data suggest that *KIT* mutations and genetic *KIT* aberrations may be of prognostic significance for melanoma patients.

Discussion

In the Caucasian population, the major subtype of melanoma is CSD (1, 2, 4, 5). In contrast, acral and mucosal melanoma, which constitute a small proportion of melanomas in Caucasians, are the most prevalent melanoma subtypes in non-Caucasians, especially in Chinese, as evidenced by our study and by the others (1, 2–7). We found that the incidence of *KIT* mutation was lower in acral and mucosal melanomas, as compared with that in CSD melanomas and to that reported by others (4, 5, 12–15). The discrepancies between our study and previous reports may be attributed to differences in pathogenesis and genetic predisposition to melanoma. Small sample sizes in previous studies and inadvertent patient selection factors may contribute to the difference. For therapeutic purposes, *KIT* aberrations may be of clinical importance in identifying patients who may benefit from small molecule inhibitors (e.g., Imatinib; refs 16, 17, 21, 22). Diverse *KIT* mutations were detected in Chinese melanoma patients, with only the K642E, L576P, V559A, and W557R mutations having known sensitivity to imatinib (5, 12–15). Although it has been suggested that mutations affecting the JMD domain will lead to gain-of-function of KIT (28, 29), the significance of the other mutations affecting the ECD and kinase domain of KIT discovered in our study awaits further evaluation. There is recent preliminary evidence provided by Carvajal and colleagues that imatinib can induce an overall response rate of 33% in the small proportion of melanomas with mutations in exon 9 and exon 13 of *KIT* gene (22). However, in our preliminary phase II clinical trial of imatinib (400 mg/day), we could only obtain an overall response rate of 21% (6/28; ref 30). We speculate that the more enrolled patients, the more variable mutations (e.g., mutations in exons other than exon 9 or exon 13) in *KIT* and the more advanced stages in our cohort may contribute to this difference (22, 30), which may suggest for a more strict selection strategy for enrollment of imitinib-sensitive melanoma patients.

KIT mutations have been screened in various tumors (23, 24, 28) and have been suggested as an adverse prognostic factor for survival (31–34). In GIST, it has been suggested that *KIT* mutation is an independent prognostic factor for overall and cause-specific survival of patients with GIST (31). In another study, *KIT* mutation and activation are important in GIST pathogenesis and may provide important prognostic information (32). In acute myeloid leukemia patients, *KIT* mutations confer higher relapse risk and appear to adversely affect overall survival (33, 34). However, the significance of *KIT* mutations in melanoma prognosis has not been evaluated in an adequately sized study. In a multivariate analysis of 13,581 patients with localized melanoma, the 2 most powerful independent characteristics of the primary melanoma among all the prognostic variables analyzed were tumor thickness and ulceration (26). Other statistically significant prognostic factors are patient age, site of the primary melanoma, level of invasion, and sex (26). In our study, we found that the ulceration and stages, but not the age, sex, or thickness, were common prognostic factors for Chinese patients. *KIT* mutations, *KIT* amplifications, and CD117 expression level appear unrelated to the age, gender, tumor thickness, stage, or ulceration of melanomas. More importantly, we found that *KIT* mutations and genetic aberrations (mutation plus amplification) of *KIT* adversely impact survival. This is the first such report of such a relationship and underscores the importance of accelerating the clinical development of KIT inhibitors in this setting.

In conclusion, our study has confirmed that acral and mucosal melanomas are the most prevalent subtypes of melanoma amongst Chinese patients. Our study further suggests that *KIT* mutations may be unrelated to CD117 overexpression, whereas the increases in *KIT* gene copy numbers may be responsible for elevated CD117 expression. We found that *KIT* aberrations are unrelated to the age, gender, stages, thickness, and ulceration of primary melanomas. Most importantly, genetic mutations in *KIT* may be an independent adverse prognostic factor in melanoma. Our study also highlights that there are numerous types of *KIT* mutations present in this population, and that the responsiveness to KIT inhibitors will almost certainly vary. Careful screening for *KIT* mutations in all exons is necessary to identify patients for inclusion in clinical trials of KIT inhibitors.

Disclosure of Potential Conflicts of Interest

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