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Frequent *EVI1* translocations in myeloid blast crisis CML that evolves through tyrosine kinase inhibitors

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Clinical variables associated with ecotropic viral integration site 1 (*EVI1*) translocations were evaluated in 42 consecutive chronic myeloid leukemia (CML) patients in myeloid blast crisis (MBC). Translocations were confirmed with fluorescence in situ hybridization, and Western blot analysis demonstrated EVI1 expression. Translocations of *EVI1* were present in 3 of 24 (12%) patients whose disease evolved MBC before tyrosine kinase inhibitor (TKI) exposure, and 7 of 18 (39%) patients who had received one or more TKIs. Univariate analysis showed that prior TKI therapy was the only clinical variable that was significantly associated with *EVI1* translocation ($P = 0.047$). TKI-resistant *BCR–ABL1* mutations were present in 71% of MBC patients with *EVI1* translocations at the time of disease progression. These observations suggest that *EVI1* overexpression collaborates with *BCR–ABL1* in the evolution of TKI-resistant MBC. Inhibition of c-ABL kinase-mediated DNA double-strand repair by TKIs may predispose to *EVI1* translocation in this setting.

Keywords *BCR–ABL1*, chronic myeloid leukemia, *EVI1*, mutation, myeloid blast crisis, tyrosine kinase inhibitor

Although tyrosine kinase inhibitors (TKIs) have markedly improved the outcomes of patients with chronic phase chronic myeloid leukemia (CML), they have been less effective against myeloid blast crisis (MBC). Imatinib induced hematologic responses in 52% of CML patients whose disease evolved MBC after interferon-α therapy, but only 32% of patients survived for 12 months (1). Dasatinib produced a 34% hematologic response rate in CML MBC after imatinib failure, and the median progression-free survival was 5 months (2). The failure of TKIs to control CML MBC is due to the frequent acquisition of *BCR–ABL1* point mutations that reduce drug activity against the target, and to the evolution of additional cytogenetic abnormalities that reduce the dependence on *BCR–ABL1* signaling.

Human ecotropic viral integration site 1 (*EVI1*), located on chromosome 3 at q26, is translocated in cases of acute myeloid leukemia (AML), high-risk myelodysplastic syndrome (MDS), and CML MBC (3). The most common recurrently observed translocations, including t(3;3)(q21;q26), inv(3)(q21;q26), and t(3;21)(q26;q22), result in *EVI1* overexpression and are not disease specific. EVI1 can inhibit differentiation by binding to myeloid transcription factors, such as PU.1, and interfering with their function (4,5). EVI1 also binds DNA in a sequence-specific manner, and its overexpression is associated with hypermethylation of many gene promoter regions in AML cells, suggesting a wide-ranging effect of EVI1 on cell biology (6).

This study reports an association between prior TKI exposure and *EVI1* translocations in a sequential cohort of patients with CML in MBC.

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Methods

Patients and samples

Forty-two consecutive MBC patients enrolled onto clinical trials of TKIs were studied. The 24 patients enrolled onto imatinib trials had disease that evolved MBC before TKI exposure, and the remaining 18 patients received one or more TKIs before MBC (Table 1). All patients signed an informed consent form approved by the UCLA institutional review board.

Cytogenetics and fluorescence in situ hybridization

Cytogenetic studies were performed on 20 Giemsa-banded metaphase cells from each cultured bone marrow sample. For fluorescence in situ hybridization (FISH) studies, dual-color probes that straddle the *EVI1* gene at 3q26 were used (Kreatech Diagnostics, Amsterdam, The Netherlands). Rearrangements of *EVI1* were detected as split red or green signals (Figure 1A).

Western blot analysis

Proteins extracted from bone marrow or peripheral blood mononuclear cells were subjected to Western blot testing, as previously described (5). Blots were hybridized with either EVI1 antibody (C50E12; Cell Signaling Technology, Beverly, MA) or GAPDH (glyceraldehyde-3-phosphate dehydrogenase) antibody (FL-335; Santa Cruz Biotechnology, Santa Cruz, CA), then secondary antibody, as reported previously (5).

BCR–ABL1 kinase domain sequencing

Sequencing of cDNA for *BCR–ABL1* kinase domain mutations was performed as previously described (7).

Statistical analysis

We used *t*-tests and chi-square tests to explore an association between *EVI1* translocations and clinical variables including age, chronic phase duration, number of treatments, TKI exposure, and duration of TKI therapy. Multiple logistic regression evaluated an association of these factors and the presence or absence of *EVI1* translocations. Cox proportional hazard survival analysis examined the impact of clinical variables on response duration and survival time.

Results

A statistically significant association was observed between *EVI1* translocations and prior TKI exposure ($P = 0.047$), but not for patient age, chronic phase duration, number of treatments, or duration of TKI therapy by univariate analysis. Multivariate analysis did not identify any variable that strengthened this association. Seven of 18 (39%) patients treated with one or more TKIs before their disease evolved MBC had an *EVI1* translocation, compared to 3 of 24 (12%) who were TKI naive (Table 1). Two additional patients acquired an *EVI1* translocation while receiving a TKI for MBC, further strengthening this association. The other most common chromosomal abnormalities observed in our cohort, a second t(9;22) and trisomy 8, did not differ in frequency between the two groups.

BCR–ABL1 point mutations were identified in 9 of 17 (53%) patients with available sequencing results whose disease evolved MBC on a TKI, including 5 of 7 (71%) patients with an *EVI1* translocation (Table 1). In addition, both patients who developed a new *EVI1* translocation while receiving a TKI for MBC acquired *BCR–ABL1* mutations. All mutations conferred resistance to the TKI that the patient was receiving.

EVI1 translocations in this cohort were confirmed by FISH in patients with five different translocations (Figure 1A). The previously unreported t(3;4)(q26;q31) observed in patient 16N was found to harbor an *EVI1* translocation, but the t(3;4)(q27;p14) in patient 19N did not. Rearrangement of *EVI1* was not observed by FISH in four TKI-resistant MBC patients without 3q26 translocations by routine cytogenetics. There was complete agreement between cytogenetics and FISH, confirming that routine cytogenetic analysis can detect *EVI1* translocations with high sensitivity (8).

EVI1 proteins expressed by blasts from six patients with different *EVI1* translocations were evaluated by Western blot test (Figure 1B). Myeloblasts from all patients expressed the 145 and 88 kDa forms of EVI1. In addition, an 80 kDa form of EVI1 was expressed at high levels in blasts harboring the t(2;3)(p23;q26). Myeloblasts with the t(3;21)(q26;q22) expressed the RUNX1–MDS1–EVI1 fusion protein (180 kDa).

Discussion

EVI1 translocations were infrequently observed in advanced CML before the TKI era. Only 12% of study patients with MBC before TKI therapy had *EVI1* translocations. Similar results were reported for advanced-phase CML at the Fred Hutchinson Cancer Center before the availability of imatinib; rearrangements involving 3q21, 3q25, or 3q26 were reported in only 5% of 126 patients (9). In contrast, 39% of study patients whose disease evolved MBC while receiving TKI therapy had an *EVI1* translocation. At the M. D. Anderson Cancer Center, 37% of 68 CML patients whose disease evolved MBC after the availability of imatinib had *EVI1* translocations, corroborating our observations (10).

The relationship between TKI exposure and *EVI1* translocations may be due in part to TKI inhibition of the c-ABL kinase. After DNA damage, c-ABL translocates from the cytoplasm to the nucleus, where it induces cell cycle arrest and initiates DNA repair (11). c-ABL participates in homologous recombination repair of DNA double-strand breaks by binding to and phosphorylating RAD51, which oligomerizes around damaged DNA and recruits additional proteins involved in DNA repair (12,13). Inhibition of c-ABL kinase activity by imatinib or dasatinib is likely to compromise repair of double-strand DNA breaks and increase the occurrence of translocation events. This may be particularly true in cells harboring TKI-resistant *BCR–ABL1* mutations, in which the