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## IDH2 somatic mutations in chronic myeloid leukemia patients in blast crisis

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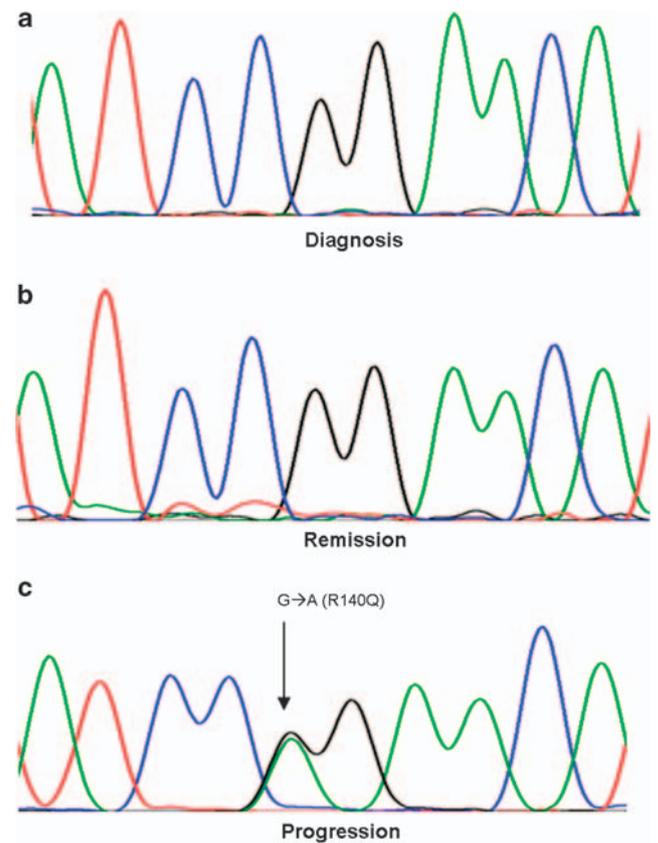
We read with interest a series of manuscripts,<sup>1–6</sup> reporting somatic mutations of the isocitrate dehydrogenase 1 and 2 enzyme isoforms (IDH1, IDH2) in patients with *de novo* acute myeloid leukemia (AML), in patients with chronic and blast phase Philadelphia chromosome-negative (Ph<sup>-</sup>) myeloproliferative neoplasms (MPNs) and in patients with early and accelerated phase myelodysplastic syndromes (MDSs).

We have recently screened, by massively parallel sequencing, the transcriptome of a Philadelphia chromosome-positive (Ph<sup>+</sup>) chronic myeloid leukemia (CML) patient. The patient was a 62-year-old female diagnosed with Ph<sup>+</sup> p210<sup>BCR-ABL</sup>-positive CML, high risk according to both Sokal (2.4) and Euro (1704.39) scores. No additional chromosomal abnormalities were detected at diagnosis by chromosome banding analysis. The patient was enrolled on a phase 2 study of nilotinib 400 mg twice daily as first line treatment of CML.<sup>7</sup> She achieved a major molecular response (BCR-ABL/ABL = 0.1% according to the International Scale) after 3 months, but suddenly progressed to lymphoid blast crisis (BC) after 6 months from diagnosis. At that time, mutation screening of the Abl kinase domain performed by Sanger sequencing showed evidence of a nilotinib-resistant T315I mutation, whereas no additional chromosomal abnormalities were detectable. The patient died of her disease 1 month, thereafter. After having obtained written informed consent from her next of kin, we retrieved the samples collected at diagnosis, at the time of major molecular response and at the time of disease progression, isolated messenger RNA and proceeded to massively parallel sequencing on a Solexa Illumina Genome Analyzer II platform according to manufacturer's instructions.

An IDH2 R140Q heterozygous mutation deriving from a G to A nucleotide substitution on chromosome 15, position 88432938 (hg18, NCBI build 36.1) was found in the sample collected at the time of progression to lymphoid BC, but this variant was not seen in the sample collected at the time of diagnosis nor in the sample collected at the time of remission. Conventional Sanger sequencing performed on genomic DNA confirmed massively parallel sequencing findings (Figures 1a–c). Other sequence changes were also detected that will be reported in detail elsewhere.

A number of recent studies have reported this same IDH2 R140Q mutation in patients with various forms of leukemia, but data in CML and acute lymphoblastic leukemia (ALL) were still lacking. This prompted us to investigate the frequency of IDH1 and IDH2 mutations in a large series of CML and ALL patients. IDH1 and IDH2 exon 4, where all the mutations so far

reported map, were screened by direct sequencing in 50 patients with newly diagnosed chronic phase (CP) CML, 5 patients with accelerated phase CML, 30 patients with lymphoid BC CML, 30 patients with myeloid BC CML, 5 patients with mixed myeloid/lymphoid BC CML, 34 patients with Ph<sup>+</sup> ALL and 23 patients with Ph<sup>-</sup> ALL (Table 1) (methods detailed in Supplementary Information), but no mutations were found. Given



**Figure 1** Presence of the R140Q IDH2 mutation at the time of progression to blast crisis (BC) (c), but neither at the time of diagnosis (a) nor at the time of remission (b). In the original lymphoid BC patient sample, analyzed by massively parallel sequencing, conventional Sanger sequencing confirmed that the G to A nucleotide substitution at position 88432938 (hg18, NCBI build 36.1), corresponding to the R140Q mutation, was present in the sample collected at the time of disease progression, but not before. Unfortunately, for the three additional myeloid BC patients found to harbour the same R140Q mutation, no paired chronic phase samples were available to confirm that, similar to the case shown here, the mutation was associated with disease progression.

**Table 1** Summary of IDH1 and IDH2 mutation frequency in CML, Ph+ ALL, Ph- ALL as assessed in our study

	IDH1	IDH2
Total patients analyzed	170	227
<i>CML</i>		
CP	0/50	0/50
AP	0/5	0/5
myBC	0/75	3/75 (4%)
lyBC	0/31	1/31 (3.2%)
mixed my/ly BC	0/9	0/9
Ph + ALL	N.A.	0/34
Ph - ALL	N.A.	0/23

Abbreviations: ALL, acute lymphoblastic leukemia; AP, accelerated phase; BC, blast crisis; CML, chronic myeloid leukemia; CP, chronic phase; IDH, isocitrate dehydrogenase; ly, lymphoid; my, myeloid.

that we originally detected the IDH2 mutation in a BC CML patient, we decided to further increase the number of BC CML patients analyzed by including an additional subset of 49 cases (45 with myeloid BC, 4 with mixed myeloid and lymphoid BC). Patients were screened by high-resolution melting analysis. Again, no mutations were detected in the *IDH1* gene, whereas an *IDH2* abnormal melting profile was detected in three cases with myeloid BC. The presence of a heterozygous R140Q mutation was subsequently confirmed by Sanger sequencing both at the DNA and at the RNA level (Table 1). Unfortunately, no paired CP samples were available to confirm that in these three additional cases, similar to the first one, the mutation was associated with disease progression. The four IDH2-mutated BC CML patients were further analyzed for *IKZF1* gene deletions and JAK2 mutations. The lymphoid BC CML patient turned out to be positive for the  $\Delta 4-7$  *IKZF1* gene deletion, and one of the three myeloid BC patients scored positive for the V617F JAK2 mutation.

IDH1 and IDH2 are nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>)-dependent dehydrogenases that catalyze the oxidative decarboxylation of isocitrate to  $\alpha$ -ketoglutarate in the Krebs cycle. The IDH1 R132H mutation was first detected in a whole-genome sequencing analysis of glioblastomas.<sup>8</sup> Subsequent studies uncovered a spectrum of missense mutations leading to amino acid changes at IDH1 R132 and, at a lesser frequency, at the homologous residue of IDH2 R172 in up to 80% of gliomas and secondary glioblastomas.<sup>9–15</sup> IDH1 and IDH2 mutations were mutually exclusive and always heterozygous. R132 and R172 are two homologous residues binding isocitrate in the enzyme active site, and mutations have been shown to create a neomorphic enzyme converting  $\alpha$ -ketoglutarate into 2-hydroxyglutarate.<sup>16,17</sup> From experimental observations in neuronal tissues, it was hypothesized that 2-hydroxyglutarate might function as an oncometabolite, increasing intracellular reactive oxygen species levels;<sup>18,19</sup> in addition, the concomitant consumption of NADPH during the conversion thought to interfere with the biosynthesis of glutathione, which protects cells from oxidative stress.<sup>20</sup> Very recently, an exponentially increasing number of publications have surprisingly showed that IDH1 and IDH2 mutations are also implicated in various forms of leukemia. The first reports came from studies in AML. Initially uncovered by a whole-genome sequencing survey,<sup>21</sup> the IDH1 R132C mutation was subsequently searched in large cohorts of primary AML cases<sup>1,21–27</sup> and was found to be detectable in a proportion of patients varying from 4.4 to 14%. Some of these studies also identified mutations affecting IDH2 R172 in

1.1–3.6% of cases, and a novel R140Q mutation in 6–15.6% of cases.<sup>17,22,25–28</sup>

IDH1 and/or IDH2 mutations at the three known hotspots were almost concomitantly reported, at an even higher frequency, in secondary AML developed from MPN.<sup>2,29,30</sup> A recent multi-institutional study in 1473 MPN patients has reported IDH mutation frequencies of 25% for blast phase polycythemia vera and for blast phase primary myelofibrosis, as against 1% for essential thrombocythemia, 2% for polycythemia vera and 4% for primary myelofibrosis in CP.<sup>4</sup> Similarly, IDH mutations have been detected in up to 15% of secondary AML evolved from an MDS—as against 3.6–5% of early stage MDS.<sup>3,31</sup> This striking association of IDH mutations with transformed/progressive disease in the context of MPN/MDS has further been strengthened by the results of a very recent study comparing mutation frequencies within a relatively well-defined, genetically homogeneous patient population, that is, between low-risk MDS and high-risk MDS/AML cases with isolated deletion of chromosome 5q (0 and 22%, respectively).<sup>5</sup> This, together with occasional findings of the same amino acid substitution in paired samples of chronic/acute phase MDS and MPN,<sup>2,3,31</sup> has raised the hypothesis that *IDH* gene mutations could represent (early) genetic events facilitating disease transformation.

We here suggest, for the first time, that this general principle may apply also in CML. We detected IDH mutations in the setting of both lymphoid and myeloid BC CML—but not in CP CML patients. In our original patient analyzed by massively parallel sequencing, for whom the IDH2 mutation status could sequentially be assessed at diagnosis and at progression, we could verify a specific association between development of R140Q and disease transformation to an acute phase (Figure 1), and the high sensitivity of this sequencing approach rules out the possibility that the mutation could be present at diagnosis in a very small percentage of Ph+ cells. However, progression to BC was so sudden in this patient that there were no additional samples available between the time she achieved major molecular response (3 months) and the time she relapsed with evidence of disease transformation (6 months)—so it is impossible to establish whether the IDH2 mutation was acquired at the time or shortly before progression. Nor was it possible to analyze CP samples from the three myeloid BC patients scored positive for the IDH2 mutation.

On the other hand, it is puzzling that the frequency of *IDH* gene mutations we found in BC CML (overall, 4/115 (3.5%) cases) is markedly lower than that observed in blast phase MDS or MPN (22–25%). According to our data, the development of IDH mutations in CML does not seem to be, or to be associated with, a major route of disease progression, given its low frequency. Hence, whether the acquisition of IDH1/2 mutations harbours some pathogenetic role in disease evolution, at least in CML, remains to be assessed. BC CML is characterized by a heterogeneous repertoire of additional cytogenetic alterations, which might explain the limited 'need' for IDH mutations. Our lymphoid BC patient harbouring the R140Q mutation was indeed negative for additional chromosomal abnormalities—whereas no cytogenetic data were available for the three remaining patients. However, this patient was positive for the  $\Delta 4-7$  *IKZF1* gene deletion, and the resulting expression of aberrant I $\kappa$ 6 Ikaros isoform has been hypothesized to have its own role in disease progression, given its oncogenic features and its recurrent detection in lymphoid BC.<sup>32</sup>

Our data provide further knowledge on the incidence of *IDH1/2* gene mutations in leukemias. On the other hand, they question a leading role for these mutations in progression from the chronic to the acute phase of CML, and underline the importance of a precise understanding of the biological consequences these mutations bring in leukemic cells.

## Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Leukemia website (<http://www.nature.com/leu>)

## Complete remission after blinatumomab-induced donor T-cell activation in three pediatric patients with post-transplant relapsed acute lymphoblastic leukemia

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Although post-transplant donor lymphocyte infusions might induce remissions in few patients via the induction of a graft-versus-leukemia effect,<sup>1</sup> refractory relapse of B-precursor acute lymphoblastic leukemia (ALL) after allogeneic hematopoietic stem cell transplantation (HSCT) is associated with a dismal prognosis. Morphological disease<sup>2</sup> or persistent minimal residual disease (MRD) levels of  $>10^{-4}$  leukemic blasts<sup>3</sup> before allogeneic HSCT have been shown to be associated with a high risk of relapse after transplantation and a poor outcome. Despite the use of recently introduced chemotherapeutic agents for refractory leukemia,<sup>4</sup> patients who relapse after allogeneic HSCT often have refractory disease and are particularly susceptible to chemotherapy-related toxicity.

T-cell engaging antibodies are a novel strategy for treatment of lymphoma and leukemia.<sup>5</sup> Such bispecific antibodies are designed to transiently engage primed cytotoxic effector-memory T lymphocytes for the lysis of target cells. By using the T-cell engaging CD19/CD3-bispecific antibody blinatumomab, partial and complete remissions (CRs) in patients with relapsed B cell non-Hodgkin lymphomas,<sup>6</sup> and elimination of chemorefractory residual leukemic blasts in bone marrow of the adult patients with MRD-positive B-precursor ALL were observed.<sup>7</sup> Although in these patients with B-precursor ALL, the blinatumomab-engaged cytotoxic T cells were patient derived, blinatumomab had so far not been administered in pediatric patients or in any patients after allogeneic HSCT, a condition in which the engaged T cells are donor derived.

After informed consent from the care givers and patients' assent were obtained, we treated three patients with blinatumomab, all of whom had post-transplant relapsed B-precursor ALL. The compassionate use of blinatumomab in all three patients was approved by the institutional review board and by the responsible local regulatory authority (Regierungsbezirk Oberbayern, Munich, Germany). Blinatumomab was provided by Micromet (Munich, Germany). Apart from a single prednisone dose of 2 mg/kg at the first day before the continuous intravenous infusion of blinatumomab, no other concomitant antileukemic drugs were administered.

The first patient was a 7-year-old boy, who was diagnosed in 2004 with high-risk B-precursor ALL and was treated according to the COALL protocol. He experienced a bone marrow relapse in June 2006 and was treated according to the ALL-REZ BFM 2002 protocol in the S3 arm. After two cycles of chemotherapy, the patient had persistent disease. A complete second remission was achieved after three courses of clofarabine. In February

2007, he received an allogeneic HSCT from a 9 out of 10 human leukocyte antigen (HLA) allele-matched-unrelated donor after conditioning with total body irradiation and etoposide. At 1 year after allogeneic HSCT, he experienced another bone marrow relapse and received subsequent chemotherapy including one cycle of clofarabine/cyclophosphamide/etoposide, two cycles of amsacrine/etoposide/prednisone and one cycle of melphalan/cytarabine. At 4 weeks after the last chemotherapy cycle with melphalan (20 mg/m<sup>2</sup>) and cytarabine (2 g/m<sup>2</sup>/day  $\times$  3 days), a bone marrow aspiration revealed persistent disease with 3% leukemic blasts quantified by flow cytometry. Apart from the leukemic cell population, the residual hematopoiesis including T lymphocytes was donor derived. The patient was then treated with blinatumomab at 15  $\mu$ g/m<sup>2</sup>/day for 5 weeks by continuous intravenous infusion.

The analysis of lymphocyte populations before and during treatment with blinatumomab showed an expansion of CD3<sup>+</sup> T lymphocytes with a peak at day 8 of the treatment (Figure 1a). Concomitant analysis of peripheral lymphocytes showed persistent 100% donor chimerism. A bone marrow analysis at day 10 of treatment revealed a CR with an MRD level of  $<10^{-4}$ , which is below the quantitative detection limit. Bone marrow analysis at the end of the treatment confirmed the absence of leukemic blasts. The analysis of MRD before, during and after the treatment with blinatumomab is depicted in Figure 2a. Apart from initial transient and completely reversible mild ataxia within the first 5 days of treatment (NCI Common Terminology Criteria for Adverse Events (CTCAE)<sup>9</sup> 2), no other side effects were observed. Despite the impressive expansion of donor-derived allogeneic T lymphocytes, no signs of graft-versus-host disease were seen. In September 2008, 2 weeks after the end of treatment with blinatumomab and while in MRD-negative CR, the patient underwent a second allogeneic HSCT from his haploidentical mother using a non-myeloablative preparative regimen consisting of clofarabine, thiotepa and melphalan.<sup>8</sup> As of August 2010, 23 months after haploidentical transplantation, the patient remains in continuous MRD-negative CR.

The second patient was diagnosed with B-precursor ALL at the age of 12 and treated according to the national protocol ALL-BFM 2000 medium risk group. At 32 months later, he experienced a bone marrow relapse and received intensive multidrug chemotherapy according to the ALL-REZ BFM 2002 protocol. Because of the persistent MRD after salvage induction therapy ( $>10^{-3}$ ), allogeneic HSCT was performed from a 10 out of 10 HLA allele-matched unrelated donor after conditioning therapy with total body irradiation and etoposide. At 21 months later, the patient experienced a second bone marrow relapse. At 11 months after successful remission induction, he experienced