IDH1 R132H mutation is a rare event in myeloproliferative neoplasms as determined by a mutation specific antibody

Myeloproliferative neoplasms (MPN) are clonal hematopoietic disorders characterized by proliferation and hyperplasia of maturing myeloid cells in bone marrow (BM). Characteristic genetic alterations are found in the majority of MPN patients, including BCR-ABL1 fusion in all chronic myelogeneous leukemia (CML) mutations in Janus kinase 2 (*JAK2*) present in 90% of polycythemia vera (PV) and in 50-60% of both patients with essential thrombocytosis (ET) and primary myelofibrosis (PMF).[1] Approximately 5% of patients with PV or ET and 15-30% of patients with PMF[2] transform to acute myeloid leukemia (AML). Interestingly, the *JAK2* mutation is frequently lost in the transformed leukemic blasts. However, many MPN are wild type for the above mentioned genes and genetic events leading to MPN are unknown.

The R132H mutation in *IDH1* encoding cytosolic NADP+-dependent isocitrate dehydrogenase 1 is very frequent in human glioma diffuse astrocytoma and oligodendroglioma,[3] common in AML,[4] infrequent in myelodysplastic syndrome,[5] but extremely rare in other solid tumors. So far, all *IDH1* mutations in gliomas affect codon 132 and more than 90% of the mutations are of the R132H type. In contrast, just over half of the detected *IDH1* mutations in AML were of the R132C type, followed by R132H mutations while the other exchanges are rare. IDH1 mutated protein produces 2-hydroxyglutarate (2-HG). However, the role of 2-HG in tumor initiation and growth is not understood.[6-7] Recently, somatic *IDH1* mutations have been found using direct sequencing in AML secondary to preexisting MPN, but not in chronic

Table 1. Characteristics of IDH1 R132H positive patients.

Variable	Case A1	Case A2	Case B	Case C
MPN type	PMF	PMF	ET	ET
JAK2 V617F status	wt	mut	mut	wt
Age at diagnosis (years)	76	78	61	63

ET: essential thrombocytosis; PMF: primary myelofibrosis; ND: not done.

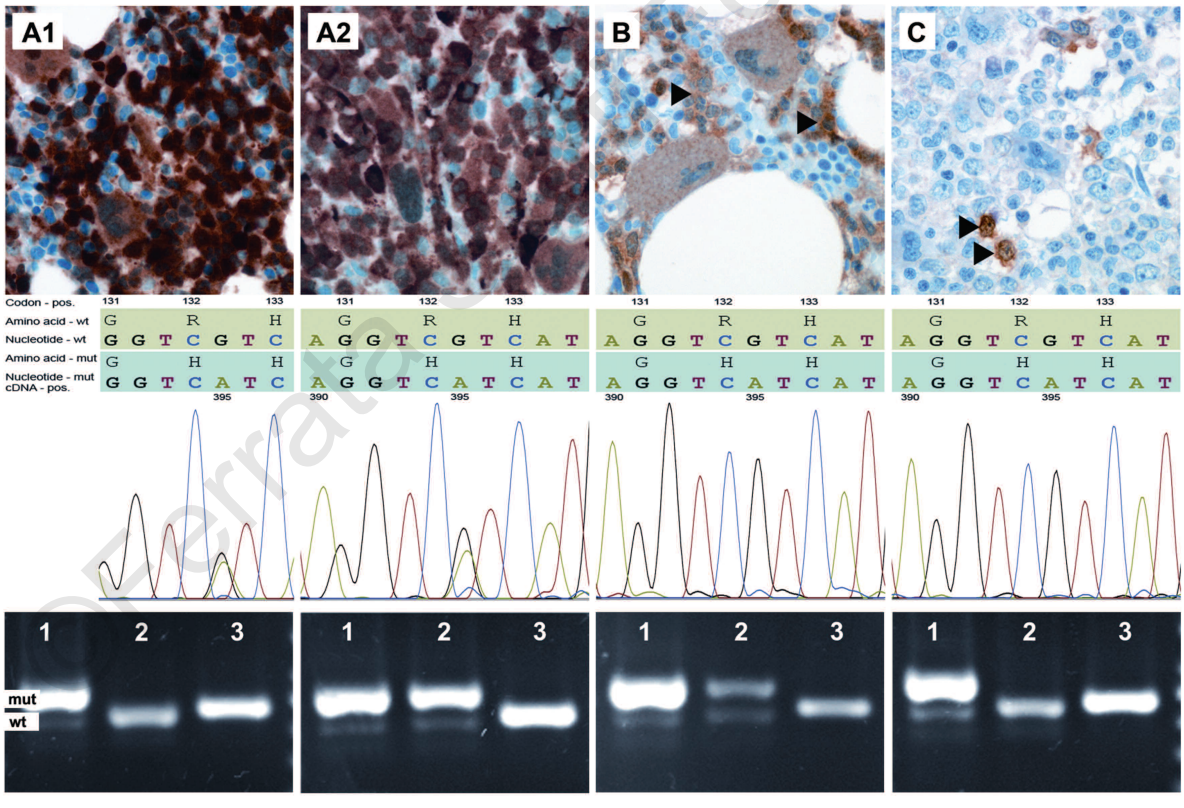

Figure 1. Detection of *IDH1*^{R132H} mutation in myeloproliferative neoplasms. BM biopsies of MPN cases stained with *IDH1*^{R132H} mutation specific antibody (upper row; magnification x400; colors corrected with Adobe Photoshop), corresponding *IDH1* DNA sequences (middle row; the position of IDH1 R132 mutated base and corresponding amino acid exchange is indicated by the DNA and amino acid sequences above) and amplification products of normal and V617F-mutant alleles of JAK2 (lower row) assessed using the amplification refractory mutation system (ARMS) as previously described;[10] lane 1 – positive control for *JAK2*^{V617F} with mut/wt allele ratio of >90%; lane 2 – the *JAK2*^{V617F} ARMS product of our corresponding cases; lane 3 – b-actin control of our corresponding cases; the products of mutated allele and wt allele are indicated by mut and wt. Almost all hematopoietic cells demonstrate strong binding of the antibody in consecutive bone marrow biopsies from case A taken at diagnosis (A1) and two years later (A2) and the R132H mutation is detectable by direct sequencing in both lesions. In contrast, the *JAK2*^{V617F} allele is not detectable in the bone marrow biopsy taken at diagnosis (A1) whereas *JAK2*^{V617F} allele is dominant in the follow-up biopsy (A2). In cases B and C, the antibody binding is evident in few positive cells (arrowheads) but no R132H mutation is found by direct sequencing.

phase of MPN suggesting a role of *IDH1* mutation in conversion from chronic MPN to acute leukemia.[8-9]

In order to test whether MPN also carries *IDH1* mutations, we investigated 160 BM biopsies of MPN patients, including CML (n=13), ET (n=73), PV (n=33), PMF (n=35) and unclassifiable MPN (n=6) using the IDH1^{R132H} mutation specific antibody. We found 2 ET and one PMF case with positive hematopoietic cells (Table 1). Thus *IDH1* mutations occur not only in AML but can also be infrequently found in the chronic MPN.

IDH1^{R132H} was detectable in the cytoplasm of granulocyte precursors, megakaryocytes and single erythroblasts. The number of IDH1^{R132H} positive cells varied between almost 100% in case A and 1-3% in cases B and C (Table 1 and Figure 1). Sequencing the *IDH1* gene of the 3 immunohistochemically positive cases confirmed the presence of R132H mutation in case A but not in cases B and C (Figure 1). The fraction of *IDH1* mutant cells in cases B and C is below the sensitivity threshold of direct sequencing which requires the presence of approximately 20% of mutant allele. Thus our data indicate that immunohistochemistry with the mutation specific antibody is a more sensitive method for detection of bone marrow cells harboring IDH1^{R132H} when compared to direct sequencing.[11]

For case A we were able to assess the chronology of *IDH1* and *JAK2* mutations based on the analyses of two consecutive bone marrow biopsies: the first taken at the initial diagnosis and the second two years later. The IDH1^{R132H} mutation was detectable by immunohistochemistry and direct sequencing in both the initial and the recurrent lesion (Figure 1 A1, A2). In contrast, the *JAK2* V617F mutation was absent in the initial BM biopsy but detectable in the follow-up biopsy. Furthermore, in the later biopsy the majority of bone marrow cells harbor the *JAK2*^{V617F} allele (Figure 1, A2, lower row). This indicates that *IDH1* R132H and *JAK2*V617F mutations are present in the same cells and not in two different cell clones. This also clearly demonstrates that *IDH1*^{R132H} mutation, similar to *TET2* mutation,[12] can occur early in the course of MPN and precede the *JAK2* mutation. Additionally, this case shows that IDH1^{R132H} was present for more than two years in virtually all hematopoietic cells but the patient did not progress to AML.

Notably, none of IDH1^{R132H} harboring cases progressed to AML within the follow-up period of 26, 16 and 118 months for cases A, B and C, respectively. This finding indicates that IDH1^{R132} mutation alone may not be sufficient for conversion of MPN to AML.

Taken together, our data demonstrate the presence of *IDH1* R132H mutation in MPN with a lower frequency than that reported in AML. Because other *IDH2* mutations are more frequent in AML, additional studies need to be carried out in order to find *IDH1* and *IDH2* mutations in chronic phase of MPN.

Furthermore, we demonstrate that standard immunohistochemistry with antibody H9 (Dianova, Hamburg, Germany) is a sensitive and reliable method to detect IDH1 R132H mutation in MPN.

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TET2 gene is not deleted in chronic myelomonocytic leukemia: a FISH retrospective study

We read with interest the paper *TET2 gene mutation is a frequent and adverse event in chronic myelomonocytic*