circulation. Double-negative T cells observed in our study population may represent a pathophysiologically important T-cell subpopulation involved in the characteristic progressive loss of hematopoietic stem cells from the bone marrow microenvironment in AA. Related, others have previously shown that T lymphocytes are a potent source of inflammatory and regulatory cytokines, including interleukin 10 (IL-10), reported to be elevated in a subgroup of patients with severe AA and more recently in patients with ALPS.4,5 Alternatively, abnormal T-cell biogenesis is merely an epiphennomenon in AA, and perhaps in ALPS, that indicates a common pathway of dysregulation leading to autoimmunity.

In conclusion, our retrospective analysis for the first time demonstrates the existence of a sizable population of double-negative T cells in AA patients, suggesting that DNTs represent a sensitive but not necessarily specific marker of ALPS. Further investigation may demonstrate previously unrecognized overlap in pathogenesis of AA and other immune cytopenias. Clearly, our observation of peripheral expansion of γ-δ TCR–expressing DNT cells in AA warrants the prospective study of a larger cohort and may lead to additional diagnostic and therapeutic approaches.

To the editor:

**IDH mutation analysis is not suitable for the routine molecular diagnostic algorithm in myeloproliferative and myelodysplastic neoplasms**

The Janus kinase 2 V617F mutation (JAK2V617F) was the first common molecular marker for the characterization of Philadelphia chromosome–negative myeloproliferative neoplasms (Ph− MPN).1 Depending on the entity, 50% to 95% of Ph− MPN are JAK2V617F-positive.1,2 Over the past few years, several other molecular defects have been described but these aberrations are detectable only in a smaller subfraction of Ph− MPN cases (<10%), for example, myeloproliferative leukaemia virus oncogene (MPL), tet oncogene family member 2 (TET2) or additional sex combs like 1 (Drosophila) (ASXL1). However, approximately 50% of these cases are also JAK2V617F-positive,2,3 making these infrequent markers not useful for routine diagnostics.

Recently, mutations in the isocitrate dehydrogenase 1 (IDH1) and IDH2 have been reported in Ph− MPN, myelodysplastic neoplasms/syndromes (MDS) and acute myeloid leukemias.4,5,6,7 These mutant enzymes exhibit decreased affinity to isocitrate, which is decarboxylated to 2-oxoglutarate by the wild-type enzymes. Mutant IDH exhibits an aberrant catalytic activity toward α-ketoglutarate, which results indirectly in accumulation of 2-hydroxyglutarate and activation of hypoxia inducible factor 1α subunit (HIF-1α).3 The IDH mutation and JAK2V617F can occur in parallel in one aberrant hematopoietic stem cell clone.5 In Ph− MPN, the mutation frequency is 0.8% for essential thrombocytopenia (ET), 1.9% for polycythemia vera (PV), 4.2% for primary myelofibrosis (PMF),4 and 3.6% for MDS.7

The aim of our study was to evaluate the usefulness of IDH mutation analysis as an additional marker for routine molecular diagnostics of chronic stage myeloid neoplasms, particularly Ph− MPN and MDS.

DNA samples (n = 326) from total bone marrow cells were analyzed with a Pyrosequencer assay (Biotage).2 A representative number of samples from ET (n = 93), prefibrotic stage PMF (n = 72), fibrotic stage PMF (n = 52), PV (n = 46), MDS (n = 45), and nonneoplastic controls (n = 18) were evaluated. Ph− MPN and MDS samples were derived from previously characterized cohorts (99% JAK2V617F PV, 48% JAK2V617F ET and PMF).2,9 Bone marrow samples were formalin-fixed and paraffin-embedded and were retrieved from the tissue archive of the Institute of Pathology (Hannover Medical School). The retrospective analysis had been approved by the local ethics committee.

None of the 263 Ph− MPN and 45 MDS cases showed detectable IDH mutations in codons R132 and R172 in IDH1 and IDH2, respectively (Figure 1). In Ph− MPN, JAK2V617F as well as IDH[R132H] can be acquired in a small subfraction of cells and can comprise a range of 5% to 10% of mutant alleles and <5% of cells,10 respectively.2 Thus, we cannot exclude that some cases with a very low mutant allele burden or cases with other IDH aberrations were not detectable by this methodology.

A molecular marker such as JAK2V617F supports the diagnosis of a histomorphologically based Ph− MPN diagnosis and thus is very helpful in distinguishing reactive from neoplastic myeloproliferation. However, due to the low occurrence of mutations, the screening for IDH1 and IDH2 aberrations is not a
The following custom-made primer sets were used for mutant allele quantification at IDH1 codon R132 (forward 5'-tccct, reverse 5'-ccatcaccatgcctaga, 83 bp; sequencing primer-1, 5'-ccatcaccatgtgctatta, 223 bp) and IDH2 (forward 5'-tgccatcttttggggtgaag, 237 bp). Wild-type status of one control was confirmed by capillary sequencing: 5'-ggcattgtgttgtggtgaag, reverse 5'-tctccaccctggcctacc, 83 bp; sequencing primer 5'-ccatcaccatgtgctatta, 223 bp).

useful tool for the routine diagnostic algorithm in chronic stage Ph'-MPN and MDS.

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To the editor:

Competing cell clones in myeloproliferative neoplasm

Patients with classic myeloproliferative neoplasms (MPNs) share a risk of transformation from a chronic phase (CP) to blast phase (BP; ie, acute myeloid leukemia, AML). Interestingly, JAK2V617F mutation detectable in most patients in CP can become undetectable upon transformation to BP, strongly suggesting that the event leading to transformation happens in JAK2V617F-negative cell clone. Recently, other mutations (eg, TET2, ASXL1, IDH1/2, RUNX1, and RAS) have been found in MPN, some possibly with higher incidence in BP than in CP, suggesting their role in the transformative process.1,2 Here we report a case that supports these recent observations of competing cell clones in MPN.

In October 2009, a 68-year-old Hispanic male with a 4-year history of hydroxyurea-treated JAK2V617F-positive polycythemia vera (PV) presented with AML. Bone marrow evaluation showed 30% myeloblasts, diploid cytogenetics, wild-type JAK2, and codon12 (G12D) NRAS mutation (wild-type NPM1 and FLT3 tested as part of AML workup). Complete remission (CR) was achieved after 2 cycles of standard 7 + 3 (cytarabine and idarubicin) induction chemotherapy; in CR, molecular testing revealed JAK2V617F positivity (48.8%) and wild-type NRAS. Re-evaluation after 4 cycles of consolidation chemotherapy confirmed CR, again with JAK2V617F positivity (47.05%) and wild-type NRAS (Table 1). There was a partial reappearance of MPN phenotype (increase of platelet count and bone marrow hyperplastic megakaryopoiesis; Figure 1).

BP develops in 5% to 8% of PV patients within 10 years after diagnosis.6 The mechanisms responsible for transformation in MPN are not well understood. Two major hypotheses have been proposed: (1) pre-JAK2 mutated clone still exists along

References


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