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Clone wars: co-occurrence of IDH2 R140Q and R172K in myelodysplastic syndromes

Valentina Rosso, Jessica Petiti, Matteo Dragani, Giacomo Andreani, Eleonora Croce, Marco Lo Iacono, Giuseppe Saglio, Carmen Fava, Daniela Cilloni

Dear Editor,

Myelodysplastic syndromes (MDS) are a heterogeneous group of diseases characterized by a clonal and ineffective hematopoiesis with risk of transformation into acute myeloid leukemia [1]. Elderly subjects, generally unfit for intensive chemotherapy, are frequently affected by MDS and the choice of treatment requires the assessment of both disease-related factors and extra-hematologic comorbidities [2]. Clinical heterogeneity of these syndromes can be attributed to a multistep pathogenesis which involves a complex pattern of mutations responsible for dysplastic features, affecting in some cases cellular metabolism and epigenetic regulation [3, 4]. Isocitrate dehydrogenase 2 (*IDH2*) gene mutations are described in 5% of MDS patients and the most frequent are *IDH2 R140Q* and *IDH2 R172K* [5]. IDH2 enzyme is involved in the tricarboxylic acid cycle and catalyzes the conversion of isocitrate to α-ketoglutarate (αKG) in mitochondria. Mutant IDH2 enzymes acquire a neomorphic enzymatic activity reducing αKG into 2-hydroxyglutarate (2HG); 2-HG competitively inhibits α-KG-dependent dioxygenase reactions leading to aberrant DNA hypermethylation and differentiation block in myeloid precursors [5]. It is still under debate if *IDH2* mutations have to be considered as drivers in leukemogenesis rather than molecular acquisitions during leukemic transformation and if they significantly impact on prognosis [6]. Recently, a new selective inhibitor of *IDH2* mutations, enasidenib, has been approved by the US Food and Drug Administration for the treatment of patients with relapsed or refractory (R/R) *IDH2*-mutated AML [5].

Analyzing *IDH2* status at different time point of a group of 79 patients with MDS by validated Droplets Digital PCR (ddPCR) Mutation Detection Assays (#dHsaMDV2010057 for *IDH2 R140Q* and #dHsaMDV2010059 for *IDH2 R172K*, Biorad), we found *IDH2* mutations in 6 patients (7.6%) and variant allele frequencies of *IDH2 R140Q* and *IDH2 R172K* mutations were between 2.1 and 12%. Surprisingly amongst our cases, we found 3 patients harboring both *IDH2 R140Q* and *IDH2 R172K* mutations (Fig. 1).

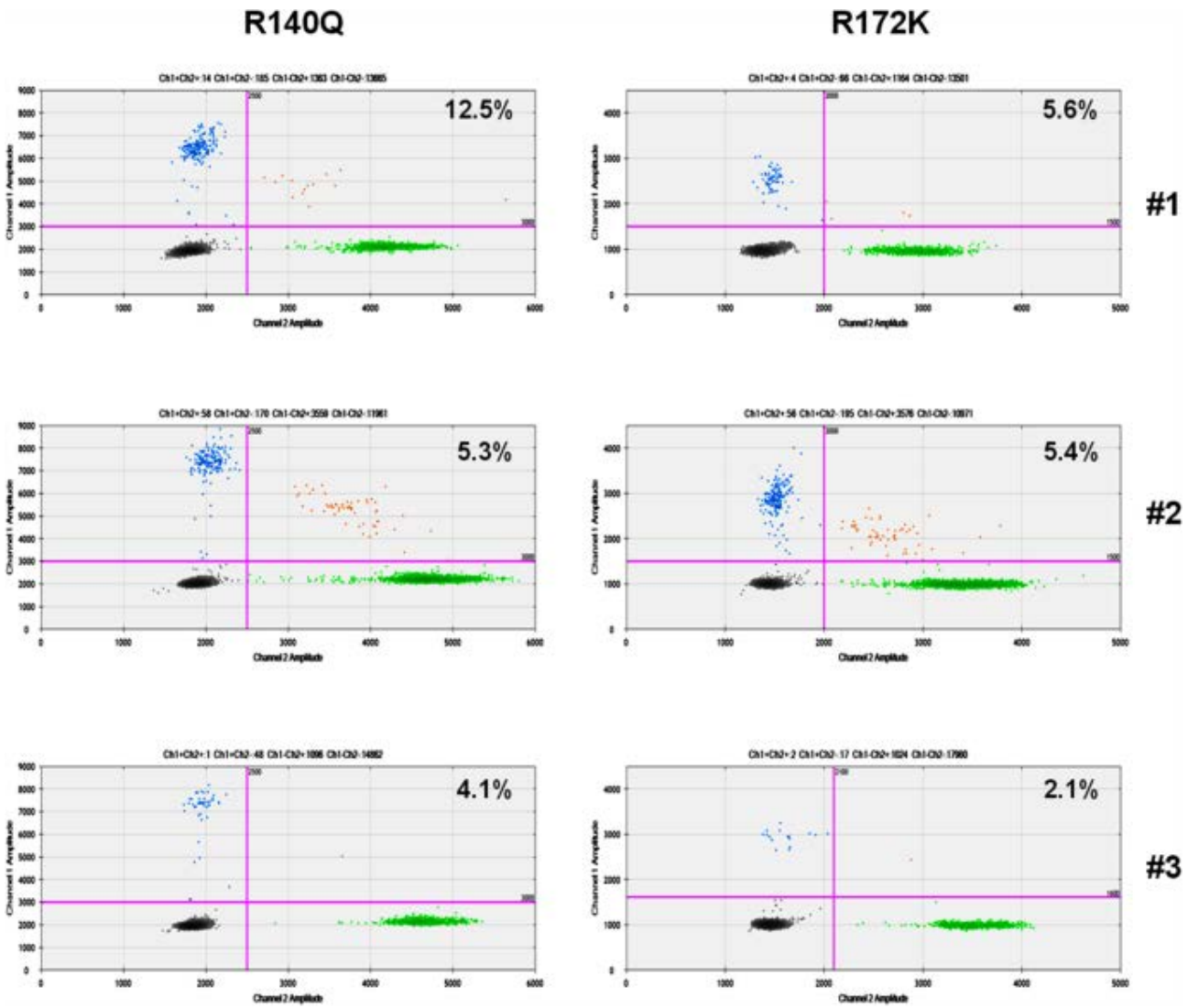

Fig. 1 2D plots of ddPCR analysis of IDH2 R140Q and R172K mutations in 3 MDS patients. For each plot, the amplitude in channel 1 (IDH2 mutated) is represented on the *y*-axis with the amplitude in channel 2 (IDH2 wild type) represented on the *x*-axis. Four clusters are identified as single-positive for IDH2 mutated (light blue) and IDH2 wild type (green), double-positive (orange) and double-negative (gray). The percentages of IDH2 mutated/IDH2 wild type were indicated in each 2D plot

Patient 1 was an 84-year-old man affected by Refractory Anemia with Excess Blasts type 1 (RAEB-1), 8% of blasts at diagnosis; because of several comorbidities, only a short course of azacitidine was attempted and rapidly discontinued for intolerance, support therapy was initiated and 18 months after diagnosis, patient died for febrile neutropenia. Patient 2 was an 81-year-old woman diagnosed with Refractory Anemia (RA) which evolved in RAEB-1 with 6% of blasts in the bone marrow, without any particular cytogenetic or molecular landmark; patient was managed with support therapy and died 12 years later for organic decay. Patient 3 was an 80-year-old man affected by Refractory Anemia with Ringed Sideroblasts (RARS), 4% of blasts at diagnosis with normal cytogenetic and molecular analysis. Four years after diagnosis, the patient evolved to overt AML with 30% of blasts. One course of azacitidine was administered but the patient died because of multiple opportunistic infections.

Recent availability of new techniques such as ddPCR has helped to better comprehend molecular landscape behind MDS, especially in scenarios where allelic burden is too low to

be caught with standard sequencing methods as happened in our cases. Furthermore, to our knowledge, this is the first time that IDH2 R140Q and IDH2 R172K mutations are reported together. The analysis of IDH2 has been performed retrospectively on samples obtained during past follow-up while both patients were already deceased. In the first patient, IDH2 R140Q and IDH2 R172K variant allele frequencies were respectively 12.5% and 5.6%, in the second patient were 5.3% and 5.4%, and in the third one, 4.1% and 2.1% respectively. To assess if both the mutations were in the same clone or in two different cell populations, we amplified and cloned the IDH2 gene from our patients in pGEM-T Easy Vector. Then, we sequenced plasmids by Sanger method using T7 and SP6 primers. We never found clones with both IDH2 mutations, but only with R140Q or R172K. These results demonstrated that IDH2 R140Q and R172K mutations were in two different clones. We can not address whether having both IDH2 R140Q and IDH2 R172K mutations and their variant allele frequencies may have a correlation with prognosis or impact on the efficacy of the oral inhibitor enasidenib. Despite this, our data suggest that patients affected by MDS with mutated IDH2 could be considered candidates for enasidenib therapy [5]. Furthermore, the presence of small clones of mutated IDH2 cells in MDS underlines the need to monitor these patients with highly sensitive technologies, such as ddPCR, to provide clinicians an important diagnostic and prognostic tool. Indeed, monitoring these clones could allow us to understand the significance of these mutations in MDS.

References

1. Savona MR, Malcovati L, Komrokji R, Tiu RV, Mughal TI, Orazi A, Kiladjian JJ, Padron E, Solary E, Tibes R, Itzykson R, Cazzola M, Mesa R, Maciejewski J, Fenaux P, Garcia-Manero G, Gerds A, Sanz G, Niemeyer CM, Cervantes F, Germing U, Cross NC, List AF, Group MMIW (2015) An international consortium proposal of uniform response criteria for myelodysplastic/myeloproliferative neoplasms (MDS/MPN) in adults. Blood 125(12):1857–1865. https://doi.org/10.1182/blood-2014-10-607341

2. Malcovati L, Hellström-Lindberg E, Bowen D, Adès L, Cermak J, del Cañizo C, Della Porta MG, Fenaux P, Gattermann N, Germing U, Jansen JH, Mittelman M, Mufti G, Platzbecker U, Sanz GF, Selleslag D, Skov-Holm M, Stauder R, Symeonidis A, van de Loosdrecht AA, de Witte T, Cazzola M (2013) Diagnosis and treatment of primary myelodysplastic syndromes in adults: recommendations from the European LeukemiaNet. Blood 122(17):2943–2964

3. Lindsley RC, Ebert BL (2013) Molecular pathophysiology of myelodysplastic syndromes. Annu Rev Pathol 8(1):21–47

4. Pellagatti A, Boultwood J (2015) The molecular pathogenesis of the myelodysplastic syndromes. Eur J Haematol 95(1):3–15

5. Dalle IA, DiNardo CD (2018) The role of enasidenib in the treatment of mutant IDH2 acute myeloid leukemia. Ther Adv Hematol 9(7):163–173

6. DiNardo CD, Jabbour E, Ravandi F, Takahashi K, Daver N, Routbort M, Patel KP, Brandt M, Pierce S, Kantarjian H, Garcia-Manero G (2016) IDH1 and IDH2 mutations in myelodysplastic syndromes and role in disease progression. Leukemia 30(4):980–984

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Author notes

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