

5. Jetani H, Garcia-Cadenas I, Nerreter T, Thomas S, Rydzek J, Meijide JB, et al. CAR T-cells targeting FLT3 have potent activity against FLT3-ITD + AML and act synergistically with the FLT3-inhibitor crenolanib. *Leukemia*. 2018;32:1168–79.
6. Li L-P, Lampert JC, Chen X, Leitao C, Popović J, Müller W, et al. Transgenic mice with a diverse human T cell antigen receptor repertoire. *Nat Med*. 2010;16:1029–34.
7. Obenaus M, Leitão C, Leisegang M, Chen X, Gavvovidis I, Van Der Bruggen P, et al. Identification of human T-cell receptors with optimal affinity to cancer antigens using antigen-negative humanized mice. *Nat Biotechnol*. 2015;33:402–7.
8. Wander SA, Levis MJ, Fathi AT. The evolving role of FLT3 inhibitors in acute myeloid leukemia: quizartinib and beyond. *Ther Adv Hematol*. 2014;5:65–77.
9. Sykulev Y, Joo M, Vturina I, Tsomides TJ, Eisen HN. Evidence that a single peptide-MHC complex on a target cell can elicit a cytolytic T cell response. *Immunity*. 1996;4:565–71.
10. Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science* (80-). 2006;314:126–9.
11. Parkhurst MR, Yang JC, Langan RC, Dudley ME, Nathan DAN, Feldman SA, et al. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. *Mol Ther*. 2011;19:620–6.
12. Morgan Ra, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg Sa. Case report of a serious adverse event following the administration of t cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther*. 2010;18:843–51.
13. Mackarehtschian K, Hardin JD, Moore KA, Boast S, Goff SP, Lemischka IR. Targeted disruption of the flk2/flt3 gene leads to deficiencies in primitive hematopoietic progenitors. *Immunity*. 1995;3:147–61.
14. DeLapeyriere O, Naquet P, Planche J, Marchetto S, Rottapel R, Gambarelli D, et al. Expression of Flt3 tyrosine kinase receptor gene in mouse hematopoietic and nervous tissues. *Differentiation*. 1995;58:351–9.
15. Manz BN, Jackson BL, Petit RS, Dustin ML, Groves J. T-cell triggering thresholds are modulated by the number of antigen within individual T-cell receptor clusters. *Proc Natl Acad Sci*. 2011;108:9089–94.

Leukemia (2019) 33:1043–1047

<https://doi.org/10.1038/s41375-018-0299-2>

Acute myeloid leukemia

IDH1-R132 changes vary according to *NPM1* and other mutations status in AML

Brunangelo Falini¹ · Orietta Spinelli² · Manja Meggendorfer³ · Maria Paola Martelli¹ · Barbara Bigerna¹ · Stefano Ascani¹ · Harald Stein⁴ · Alessandro Rambaldi² · Torsten Haferlach³

Received: 7 August 2018 / Revised: 17 September 2018 / Accepted: 26 September 2018 / Published online: 8 January 2019

© The Author(s) 2019. This article is published with open access

These authors contributed equally: Orietta Spinelli, Manja Meggendorfer, Maria Paola Martelli

These authors contributed equally: Alessandro Rambaldi, Torsten Haferlach

Supplementary information The online version of this article (<https://doi.org/10.1038/s41375-018-0299-2>) contains supplementary material, which is available to authorized users.

✉ Brunangelo Falini
brunangelo.falini@unipg.it

¹ The Institute of Hematology and Research Center for Hemato-Oncological diseases (CREO), University of Perugia, Perugia, Italy

² The Institute of Hematology, Ospedale Giovanni XXIII, Bergamo, Italy

³ The Munich Leukemia Laboratory, Munich, Germany

⁴ Pathodiagnostik Berlin, Berliner Referenz-und Konsultations Zentrum für Lymphoma and Hamatopathologie, Berlin, Germany

To the Editor:

Isocitrate dehydrogenase (*IDH1/2*) genes encode for ubiquitously expressed enzymes that catalyze a redox reaction that converts isocitrate to α -ketoglutarate while reducing NADP to NADPH and liberating CO₂ [1]. *IDH1* exerts his function in the cytoplasm and peroxisomes whilst *IDH2* is localized in the mitochondrial matrix [1]. When mutated, the *IDH1* and *IDH2* enzymes acquire a neomorphic activity leading to the conversion of α -ketoglutarate to D-2-hydroxyglutarate [2–4]. The latter compound acts as an oncometabolite by inhibiting the α -ketoglutarate-dependent enzymes that regulates epigenetic modeling, collagen synthesis and cell signaling [1]. *IDH1* and *IDH2* mutations are mutually exclusive with *TET2* mutations that are known to promote leukemia with a similar mechanism [5].

IDH1 gene mutations have been detected in 6.6–7.6% [6, 7] of AML patients, most frequently carrying a normal karyotype, and their presence has not been associated with prognostic relevance. They are heterozygous missense mutations confined

to a single arginine residue, R132, in the enzyme active site [1]. Five R132 mutations leading to different amino acid exchanges have been described [6, 7]: p.R132H, p.R132C, p.R132G, p.R132S, and p.R132L, with R132H being the most frequent [7]. As a whole group, the *IDH1*-R132 mutations are more frequent in cases carrying *NPM1* mutations [6, 7] but it is yet unknown how the amino acid substitution of arginine at position 132 correlates with the mutational status of *NPM1* and other mutations in AML. Here, combining molecular analyses and immunohistochemistry we demonstrate that the R132H and R132C substitutions show a different distribution pattern among AML genotypes.

We first investigated 140 AML patients with normal cytogenetics enrolled in Northern Italy Leukemia Group (NILG) multicenter clinical trial (NCT00495287), for which both molecular and immunohistochemical data were available (Supplementary Information). In all 140 patients, the results of next generation sequencing (NGS) for *IDH1* and *NPM1* mutations were blindly compared with those of immunohistochemistry on bone marrow (BM) biopsies using monoclonal antibodies against IDH1-R132H and NPM1, respectively. The antibody against the IDH1-R132H mutant was previously produced by Capper et al. [8] and extensively investigated in various kind of tumors. The antibody directed against the nucleophosmin (NPM1) [9] was generated in BF laboratory. Cytoplasmic nucleophosmin-1 expression was regarded as predictive of *NPM1* mutations [9, 10] (Supplementary Information). For all studies described below, written informed consent to examine leukemic samples was obtained in accordance with the Declaration of Helsinki and approval was obtained from Local Ethic Committee.

Molecular analyses revealed *NPM1* mutations in 71/140 (51%) cases. These findings were fully confirmed by immunohistochemistry that showed cytoplasmic NPM1 (predictive of *NPM1* mutations) (Fig. 1a, c, e) in the same 71 cases. In the remaining 69 cases, NPM1 expression was nucleus-restricted, as expected in cases with *NPM1* wild-type status [9].

Molecular analyses revealed *IDH1*-R132H mutations in 10/140 (7%) cases. Notably, these 10 cases were all *NPM1*-mutated and showed cytoplasmic NPM1 at immunohistochemistry (10/71:14%). The same 10 cases, revealed R132H mutant expression at cytoplasmic level (Fig. 1b, d, f), as expected for the cytosolic function of the enzyme [1]. At diagnosis, the percentage of IDH1-R132H-positive leukemic cells and with aberrant cytoplasmic NPM1 were comparable in 6/10 cases (representative examples are shown in Fig. 1a, b), whilst in 4/10 cases the IDH1-R132H-positive leukemic cells accounted for only a fraction of them, ranging between 3% and 70%, strongly suggesting that they represented a subclone. A representative example showing about 5–10% of IDH1-R132H-positive leukemic cells is shown in Fig. 1d.

Extended molecular analysis of the 140 cases also detected *IDH1* mutations other than p.R132H in 8/140 (6%) cases.

In particular: p.R132C in 3/140 cases (2%; 2 *NPM1*-mutated, 1 *NPM1*-wt), p.R132G in 2/140 cases (1%; both *NPM1*-mutated), and p.R132S in 3/140 cases (2%; all *NPM1*-mutated). Notably, all these eight cases were negative with the mAb specific for IDH1-R132H (Fig. 1g, h).

To further validate the above findings and extend the correlation of *IDH1*-R132 changes to other mutations, we analyzed at Munich Leukemia Laboratory another independent cohort of *IDH1*-mutated AML by comprehensive gene sequencing. Our previously described AML cohort [11] comprised 106 *IDH1*-mutated de novo AML patients, most often showing *IDH1*-R132H ($n = 44/106$; 41%) and R132C (39/106; 37%). In this study, we investigated all cases by NGS and gene scan targeting *IDH1* and *NPM1* beside 25 other genes (Supplementary Information). 62% (66/106) cases of *IDH1*-mutated patients showed also a *NPM1* mutation, 48% a *DNMT3A* mutation, 23% a *FLT3*-ITD, 16% a *NRAS* mutation, and 12% a *SRSF2* mutation (Fig. 2a; Supplementary Table 1). All other mutations occurred in <10% of cases. Therefore, we could confirm the high association of *IDH1*-R132H with *NPM1* mutations in this cohort. In fact, 39/44 (89%) *IDH1*-R132H patients showed a *NPM1* mutation, while in only 44% (27/62) of the other *IDH1*-R132 mutated patients a *NPM1* mutation occurred ($p < 0.001$) (Fig. 2a; Supplementary Table 1).

Analysis of further gene mutations and their associations showed that *IDH1*-R132H was mutually exclusive for *RUNX1* (0/44; 0%; $p = 0.001$), *SRSF2* (3/44; 7%; $p = 0.104$) and *ASXL1* (1/44; 2%; $p = 0.023$) and were less frequently mutated compared to *IDH1*-R132C mutated patients (23%, 21%, and 18%, respectively) (Fig. 2a, supplementary Table 1). These data resulted, therefore, in two different mutation patterns, differentiating *IDH1*-R132H and R132C mutated AML (Fig. 2b). While R132C shows a more s-AML like genetic, R132H shows a typical de novo AML pattern [12]. The third group of *IDH1*-mutated patients (other than R132H/C) seemed to be a mixture of both patterns (Fig. 2b). Addressing the prognostic impact of these *IDH1*-R132 variants showed a slightly worse prognostic impact of *IDH1*-R132C compared to *IDH1*-R132H-mutated patients (overall survival: 19.9 versus 24.9 months; Supplementary Figure 1).

Different co-mutation patterns for hotspots within genes has been previously described under various circumstances [13]. As an example, the *NPM1* mutation preferentially associates with *NRAS*-G12/13 but not with *NRAS*-Q61 [13]. These findings strongly suggest that the functional consequences of hotspot mutations within genes may not be equivalent. At present, no compound *NPM1*-mutated/*IDH1*-mutated mouse model has been described.

Is there any utility to have an anti-IDH1-R132-specific antibody in the NGS era? Although, molecular analyses remain the gold standard for the identification of *IDH1* mutations, immunohistochemistry may be a useful adjunct

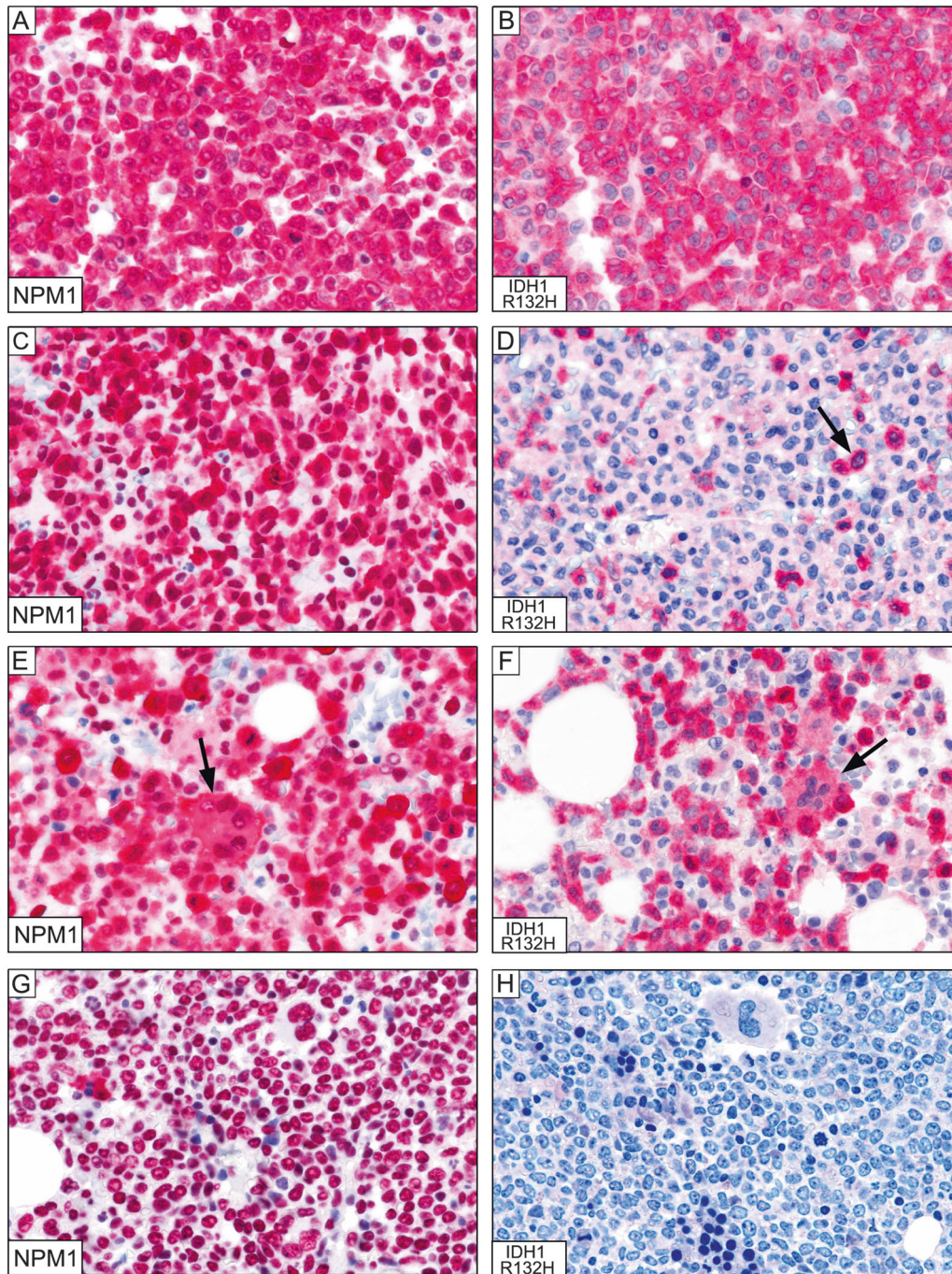


Fig. 1 **a** Massive infiltration of BM by *NPM1*-mutated AML cells showing the expected nuclear plus aberrant cytoplasmic positivity for nucleophosmin-1 ($\times 400$). **b** The same case as (**a**), showing a comparable number of leukemic cells expressing the IDH1-R132H mutant; positivity is mostly restricted to the cytoplasm of blast cells ($\times 400$). **c** Marked infiltration of BM by *NPM1*-mutated AML cells showing the expected nuclear plus aberrant cytoplasmic positivity for nucleophosmin-1. The rare elements with nucleus-restricted positivity for NPM1 represent residual normal hematopoietic cells ($\times 400$). **d** The same case as (**c**), showing that leukemic cells expressing the IDH1-R132H mutant represent only a small subclone of the total population of *NPM1*-mutated cells ($\times 400$). **e** Marked infiltration of BM by *NPM1*-mutated AML cells showing the expected nuclear plus aberrant cytoplasmic positivity for nucleophosmin-1 ($\times 400$). The arrow points

to a positive megakaryocyte. Elements with nucleus-restricted positivity for NPM1 represent normal residual hematopoietic cells ($\times 400$). **f** The same case as (**c**), showing that the percentage of leukemic cells expressing the IDH1-R132H is slightly inferior to that of NPM1 cytoplasmic-positive cells. As in (**e**), the IDH1-R132H mutant is present both in mononuclear blast cells and in a megakaryocyte (arrow). The IDH1-R132H negative cells represent normal residual hematopoietic cells ($\times 400$). **g** Massive bone marrow infiltration by leukemic cells with nucleus-restricted positivity for nucleophosmin-1 (predictive of absence of *NPM1* mutations, confirmed molecularly) ($\times 400$). **h** Specificity of the antibody against IDH1-R132H is demonstrated by the negativity of leukemic cells molecularly carrying the IDH1-R132C mutation ($\times 400$). (**a-h**) Dako REAL Detection System Alkaline Phosphatase/RED rabbit/mouse

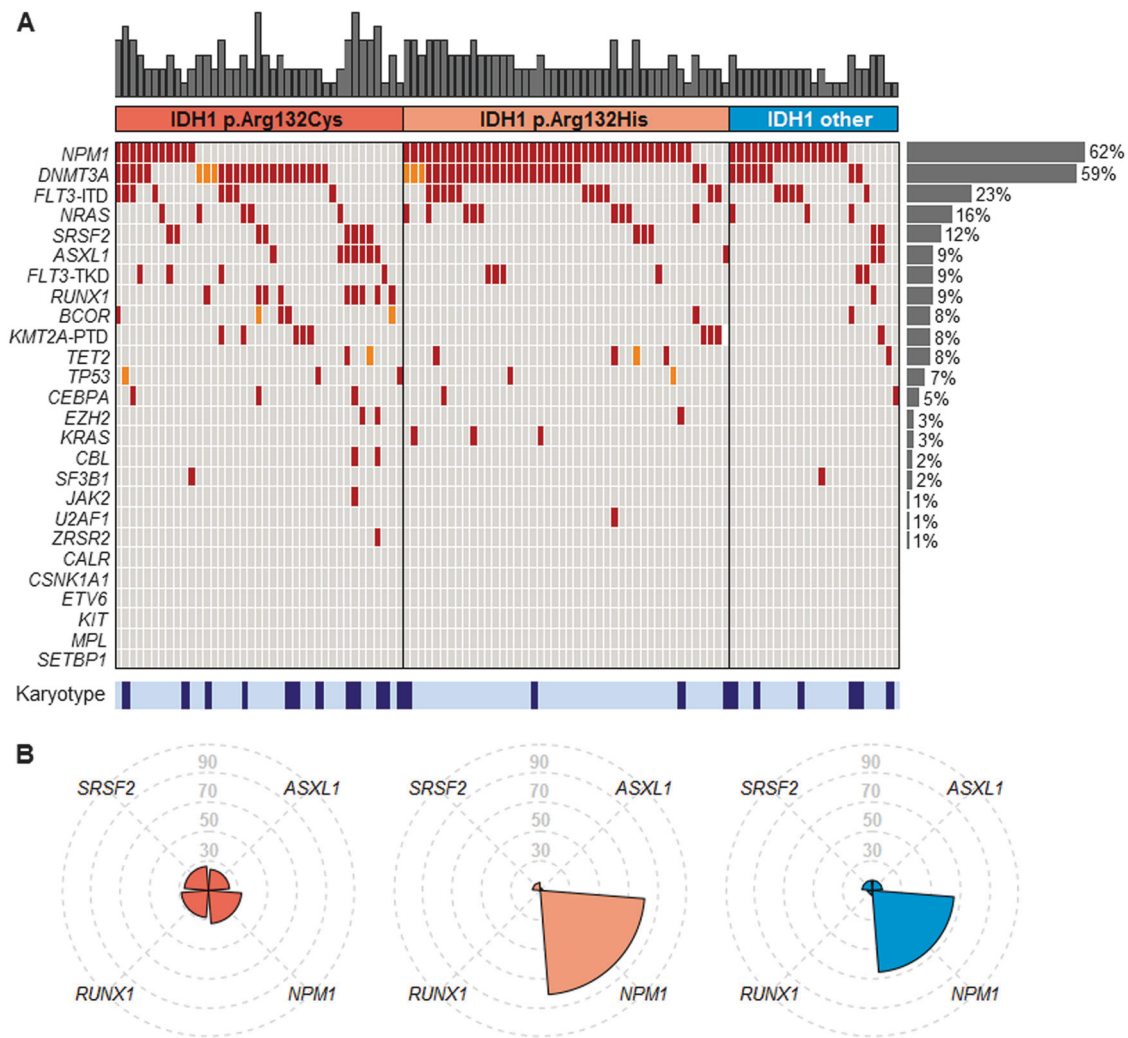


Fig. 2 a Molecular and cytogenetic characterization of *IDH1*-mutated patients. Illustration of all 106 samples, each column represents one patient. All 25 additionally analyzed genes as well as karyotype information are given for each patient. Patients are grouped by *IDH1* R132C, R132H, and R132 other. Light gray: wild type, red: mutated, orange: variant of uncertain significance, dark blue: aberrant

karyotype, light blue: normal karyotype, white: no data available. The number of additional mutations per patient is illustrated as bar chart above the graph. The mutation frequencies of single genes are given as bar chart at the right. **b** Spider plot illustrating the mutation frequencies (in %) of *ASXL1*, *NPM1*, *RUNX1*, and *SRSF2* mutations for the single groups of *IDH1* R132C, R132H, and R132 other

to the above techniques, particularly in hematological centers that still use BM biopsies. Under these circumstances, the antibody could be used both at diagnosis and for monitoring of AML after chemotherapy or targeted therapy with *IDH1* inhibitors [14]. The antibody would also allow to analyze the genetic lesion at protein level in the tissues and provide information related to the topographical distribution (nearby trabeculae or vessels) of leukemic cells. Moreover, the use of the antibody may be particularly important in cases of “punctio sicca” or myeloid sarcoma, especially when scarce material is available for molecular analyses (e.g. punch biopsies of the skin).

Acknowledgements Supported by the ERC Adv Grant 2016 no. 740230 to B.F. and the Associazione Italiana Ricerca Cancro (AIRC) IG 2016 no.18568.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Cairns RA, Mak TW. Oncogenic isocitrate dehydrogenase mutations: mechanisms, models, and clinical opportunities. *Cancer Discov.* 2013;3:730–41.
- Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Collier HA, et al. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. *Cancer Cell.* 2010;17:225–34.
- Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature.* 2009;462:739–44.
- Gross S, Cairns RA, Minden MD, Driggers EM, Bittinger MA, Jang HG, et al. Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. *J Exp Med.* 2010;207:339–44.
- Figuerola ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell.* 2010;18:553–67.
- Schnittger S, Haferlach C, Ulke M, Alpermann T, Kern W, Haferlach T. IDH1 mutations are detected in 6.6% of 1414 AML patients and are associated with intermediate risk karyotype and unfavorable prognosis in adults younger than 60 years and unmutated NPM1 status. *Blood.* 2010;116:5486–96.
- Paschka P, Schlenk RF, Gaidzik VI, Habdank M, Kronke J, Bullinger L, et al. IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication. *J Clin Oncol.* 2010;28:3636–43.
- Capper D, Zentgraf H, Balss J, Hartmann C, von Deimling A. Monoclonal antibody specific for IDH1 R132H mutation. *Acta Neuropathol.* 2009;118:599–601.
- Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med.* 2005;352:254–66.
- Falini B, Martelli MP, Bolli N, Bonasso R, Ghia E, Pallotta MT, et al. Immunohistochemistry predicts nucleophosmin (NPM) mutations in acute myeloid leukemia. *Blood.* 2006;108:1999–2005.
- Meggendorfer M, Cappelli LV, Walter W, Haferlach C, Kern W, Falini B, et al. IDH1R132, IDH2R140 and IDH2R172 in AML: different genetic landscapes correlate with outcome and may influence targeted treatment strategies. *Leukemia.* 2018;32:1249–53.
- Lindsley RC, Mar BG, Mazzola E, Grauman PV, Shareef S, Allen SL, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood.* 2015;125:1367–76.
- Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med.* 2016;374:2209–21.
- DiNardo CD, Stein EM, de Botton S, Roboz GJ, Altman JK, Mims AS, et al. Durable remissions with ivosidenib in IDH1-mutated relapsed or refractory AML. *N Engl J Med.* 2018;378:2386–98.

Leukemia (2019) 33:1047–1051

<https://doi.org/10.1038/s41375-018-0320-9>

Lymphoma

Dose-adjusted EPOCH plus rituximab improves the clinical outcome of young patients affected by double expressor diffuse large B-cell lymphoma

A. Dodero¹ · A. Guidetti^{1,2} · A. Tucci³ · F. Barretta⁴ · M. Novo⁵ · L. Devizzi¹ · A. Re³ · A. Passi³ · A. Pellegrinelli⁶ · G. Pruneri^{2,6} · R. Miceli⁴ · A. Testi⁶ · M. Pennisi¹ · M. C. Di Chio¹ · P. Matteucci¹ · C. Carniti¹ · F. Facchetti⁷ · G. Rossi³ · P. Corradini^{1,2}

Received: 17 July 2018 / Revised: 5 October 2018 / Accepted: 6 November 2018 / Published online: 10 January 2019

© The Author(s) 2019. This article is published with open access

These authors contributed equally: A. Dodero, A. Guidetti

Supplementary information The online version of this article (<https://doi.org/10.1038/s41375-018-0320-9>) contains supplementary material, which is available to authorized users.

✉ A. Guidetti
anna.guidetti@istitutotumori.mi.it
anna.guidetti@unimi.it

¹ Department of Hematology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy

² Department of Oncology and Hemato-Oncology, University of Milano, Milano, Italy

³ Department of Hematology, Azienda Ospedaliera Spedali Civili di Brescia, Brescia, Italy

To the Editor:

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease. Patients carrying the double expression of

⁴ Department of Clinical Epidemiology and Trial Organization, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy

⁵ Department of Hematology, Azienda Ospedaliero Universitaria Città' della Salute e della Scienza di Torino, Torino, Italy

⁶ Department of Pathology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy

⁷ Department of Pathology, Azienda Ospedaliera Spedali Civili di Brescia, Brescia, Italy