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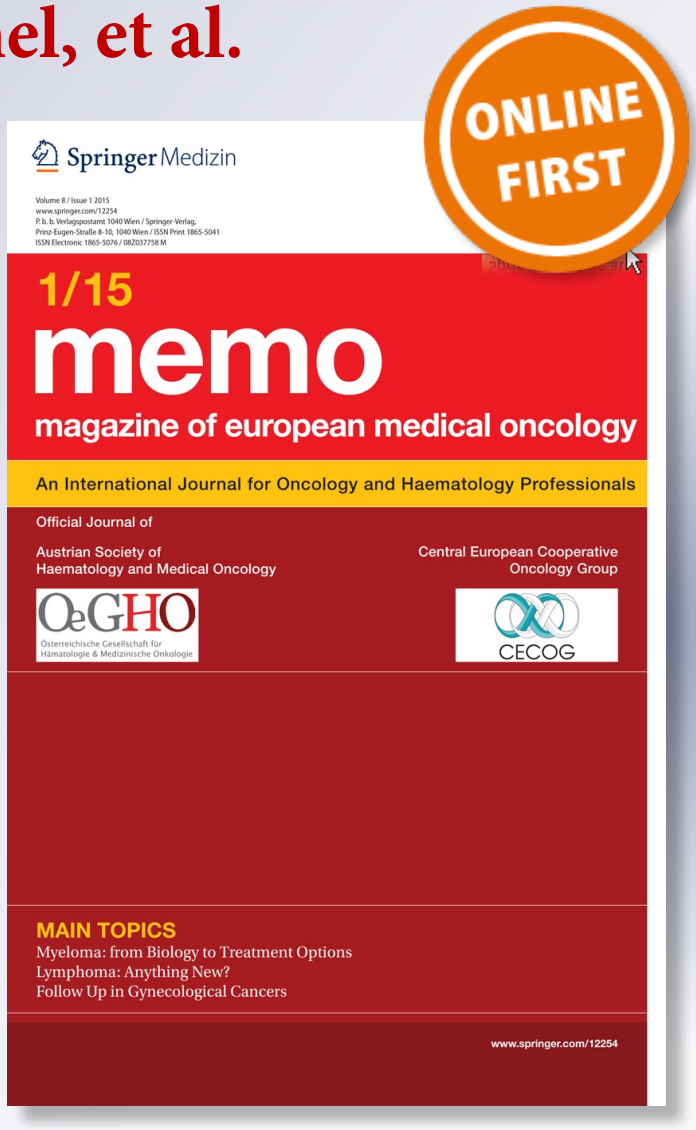

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Potential impact of the hypomethylating agent 5-azacitidine on chronic lymphocytic leukemia with del(17)(p)/del(p53) and subsequent therapy-related acute myeloid leukemia without these aberrations: a case report

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Abstract The patient presented here had a very poor prognosis due to previous chronic lymphocytic leukemia with unfavourable cytogenetics (deleted 17p and p53), therapy-related acute myeloid leukemia (intact 17p/p53), multiple pre-treatments (fludarabine, rituximab-CHOP, rituximab-bendamustine), advanced age (71 years) and several comorbidities—circumstances that usually do not allow high-dose chemotherapy or stem cell transplantation. Due to this unfavourable condition and reportedly poorer outcome of therapy-related acute myeloid leukemia relative to de novo acute myeloid leukemia with chemotherapy, treatment with the hypomethylating agent 5-azacitidine (Vidaza®), 75 mg/m²/day, subcutaneously for 7 days of 28-day cycles was started. Therapy was well tolerated and yielded a good response not only by eradicating the therapy-related acute myeloid leukemia but also by keeping the chronic lymphocytic leukemia under control for a rather long period of time. The simultaneous suppression and re-appearance of both the therapy-related acute myeloid leukemia with intact p53 and the chronic lymphocytic leukemia with deleted p53 suggests that hypomethylation by azacitidine attains tumour control by a mechanism that (i) is similar in the different tumours and (ii) acts independently of the p53 status. This case may serve as a model for disease progression mirrored in molecular and cytogenetic findings which are factored into treatment decisions.

Keywords Chronic lymphocytic leukemia · Therapy-related acute myeloid leukemia · Unfavourable cytogenetics · Poor prognosis · 5-Azacitidine

Introduction

While conventional prognostic approaches for chronic lymphocytic leukemia (CLL) consider disease stage to be the most relevant prognostic factor for patient's survival and therapy decision, today molecular markers (e.g. deletion of 17p, 11q, aberration of p53) gain increasing importance in this context. Their impact on outcome is arguably independent of standard antineoplastic treatment [1–4]. Meanwhile new drugs are becoming available approaching particularly this group of patients [5, 6]. In rare cases, pre-treated CLL patients develop therapy-related myelodysplastic syndromes (t-MDS) or therapy-related acute myeloid leukemia (t-AML) [7]. Also these conditions can be accompanied by molecular aberrations (e.g. abnormalities of chromosomes 5 and 7, aberration of p53) that when present, are associated with a poorer prognosis [7, 8]. Hence, therapy decision for both CLL and therapy-related myeloid neoplasms (t-MN) should also include a thorough analysis of molecular markers.

Here we present the case of a 71-year-old male patient with CLL and poor cytogenetics undergoing different subsequent treatments and eventually developing t-AML that was successfully treated with the hypomethylating agent 5-azacitidine. Regular analyses of molecular and cytogenetic markers throughout the entire observational period allowed us not only to mirror disease progression but also guided us in our treatment decisions.

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Case presentation

In 2002, a 71-year-old male patient with lymphocytosis without nodal manifestations was diagnosed with CLL clinical stage Binet stage A based on immunology (no molecular analyses performed). After watch-and-wait for 1 year, a rapid increase in leucocytes was treated with four cycles of oral fludarabine after confirmation of diagnosis by bone marrow analysis. Pre-treatment fluorescent in situ hybridization (FISH) analysis for a CLL panel including 17p deletion (del(17)(p)) revealed no clonal aberrations.

Another 15 months later, splenomegaly developed with slight thrombocytopenia and a renewed increase in white blood cell counts, corresponding to Binet stage B. The patient received six cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone (R-CHOP) resulting in normal blood counts and reduction in spleen size, lasting for 7 years. Then, spleen size increased again and the patient became anaemic with need of transfusions. Bone marrow testing confirmed infiltration by CLL. At this time, FISH for p53 revealed a del(17)(p) in 30 % of the cells.

Due to advanced age and cardiac comorbidity as well as lack of drugs targeting the B-cell receptor signalling pathway at that time, we chose rituximab-(R)-bendamustine as third-line treatment of CLL. This led to reduction of spleen size and moderate improvement of anaemia after five cycles. In the subsequent 5 months of watch-and-wait, anaemia worsened again and neutropenia developed. Surprisingly, a bone marrow evaluation revealed no cytomorphological signs of CLL.

Although the CLL was apparently eradicated, dysplasia and increased myeloid blasts (> 20 %) were observed; t-AML was diagnosed. FISH of the bone marrow no longer revealed del(17)(p). As the patient refused a second bone marrow aspiration, no metaphases were available to exclude further chromosomal aberrations beyond the MDS panel tested by FISH. Treatment of t-AML was started with the hypomethylating agent 5-azacitidine (Vidaza®), 75 mg/m²/day, subcutaneously for 7 days of 28-day cycles [9]. Blood counts improved; it was possible to stop red blood cell transfusions after cycle four; persistent haemoglobin levels > 12 g/dl were observed after cycle six. Platelet supplementation was never necessary. To treat neutropenia, granulocyte-colony stimulating factor was given intermittently up to cycle eight. Bone marrow reassessment after six cycles revealed complete remission without evidence of AML or CLL by cytomorphology and histology. Complete staging showed no nodal manifestations. Continuing azacitidine up to cycle 12, the patient enjoyed good quality of life with normal blood counts 11 years after initial diagnosis.

Then, the patient again became thrombocytopenic and neutropenic. Bone marrow assessment showed relapse of both neoplasms: t-AML with borderline blast counts and discrete infiltrates of CLL. In FISH analysis (suspension), del(17)(p) was detectable in approximately 50 % of the nuclei (Fig. 1), though it was not possible to identify whether the deletion was a feature of one or both tumour types. p53 expression was found by immunohistochemistry in nodal bone marrow infiltrates of CLL (Fig. 2). Azacitidine was stopped and the patient monitored with the intention to begin supportive care as needed. Seven weeks later platelet support had to be started, 8 weeks later red blood cell transfusions in addition. Cardiac comorbidities—valvular disease—became the most prominent symptom. Surgery was discussed but was not possible due to the underlying blood disorder. The thrombopoietin-receptor agonist eltrombopag (Revolade®) [10] was started on a compassionate use basis but did not lead to an increase in platelets. Finally,

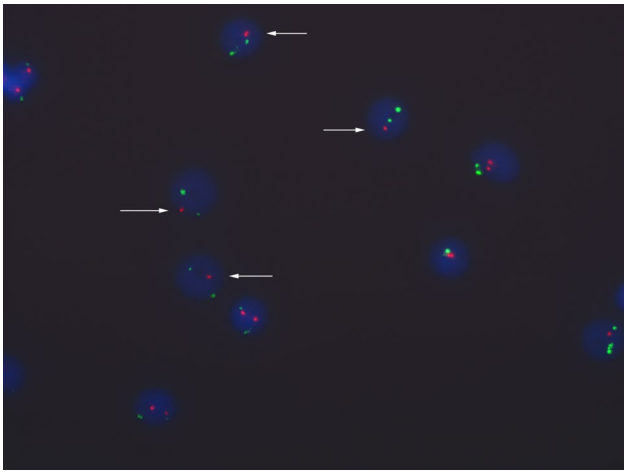

Fig. 1 Deletion of 17p as demonstrated by fluorescent in situ hybridization (FISH) in bone marrow aspirates. Normal nuclei show two green centromeric spots representing two copies of chromosome 17 and two red spots for 17(p13.1)/p53. The probe specific for p53 indicates the loss of this region in one of the chromosomes (*one red signal/cell*) (*arrows*). Blue staining (*DAPI*) represents nuclear chromatin

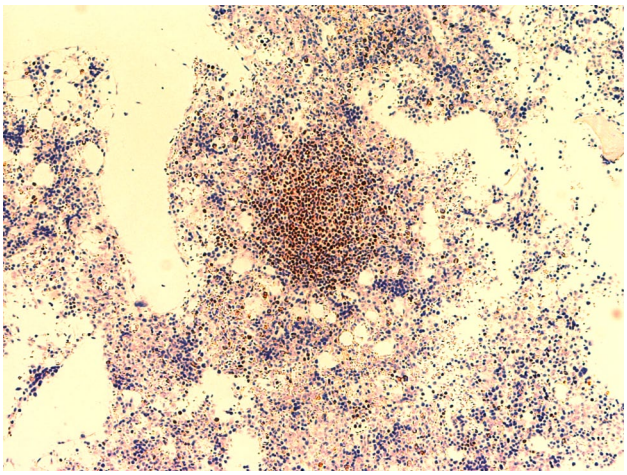

Fig. 2 Immunohistochemistry for p53 (Dako Monoclonal Mouse Anti-Human p53 Protein, Clone DO-7). Bone marrow biopsy of a nodular infiltrate of the chronic lymphatic leukemia shows strong nuclear positivity for p53

the patient died due to cardiac insufficiency during neutropenic infection.

Discussion

Initially, our CLL patient presented without del(17)(p). Approximately 9 years later and after repeated chemotherapies (fludarabine monotherapy and R-CHOP), FISH revealed del(17)(p) in the recurring CLL. Although our analyses did not specifically determine the mutational status of p53, the fact that this gene is localized on chromosome 17p and the use of a p53c-specific FISH probe suggested the presence of dysfunctional p53. After therapy with R-bendamustine, cytomorphology no longer revealed any evidence of CLL. However, t-AML was diagnosed. Although we cannot rule out that R-bendamustine caused the CLL eradication, we find it more plausible that the emerging t-AML displaced the CLL clones. FISH analysis showed no 17p/p53 deletion in the t-AML, suggesting that CLL and AML had developed from two independent clones.

t-MN may arise after alkylating agents, topoisomerase-II-inhibitors, ionizing radiation, antimetabolites and antitubulin agents [7, 11]. Our patient had received the antimetabolite fludarabine and as alkylating agents cyclophosphamide and bendamustine. With these agents t-MN is often accompanied by abnormalities of chromosome 5 and 7 and molecular aberrations such as mutation of p53 [7, 8], all of which associated with an adverse prognosis [8]. In our case, t-AML developed without such aberrations. This would have permitted the use of a cytotoxic therapy, but we decided to start with the hypomethylating agent 5-azacitidine [9] based on patient-related (comorbidities, advanced age, general condition) and disease-related (reportedly poorer outcome of t-AML relative to de novo AML with chemotherapy [12]) considerations. At that time, the hypomethylating agent 5-aza-deoxycytidine had not been approved for this indication in Europe [13]. With good tolerability azacitidine eradicated the t-AML in our patient.

While at the time of treatment no data were available on the use of azacitidine for therapy of t-AML, meanwhile some smaller studies have been published on azacitidine for t-MN [14–16]. The median length of treatment in all studies was 4.0–4.5 cycles. Overall response rates ranged from 39 to 43 % and median overall survival (OS) from 72 % at 8.5 months to 8 % after 3 years. Disease progressed in 25–27 % of the patients. Our patient had received a total of 12 cycles of azacitidine. Complete remission was achieved after six cycles; with continuing azacitidine therapy, the time to progression was 12 months. Hence, our results were in the range of the published data.

During azacitidine therapy, the CLL remained suppressed for a rather long period (approximately 1 year). Up to now, only very preliminary data on the use of azacitidine for therapy of CLL are available from one small phase II study [17]. Results from an ongoing, more comprehensive study (http://clinicaltrial.gov/ct2/show/NCT00413478?term=nct00413478&rank=1) are not yet available. However, based on reports that patients (not CLL but MDS/AML) with cytogenetic aberrations respond significantly better to azacitidine than to DNA-interfering agents [18, 19], we consider azacitidine potentially effective also for the ongoing control of the 17p-/p53-deleted CLL.

This would mean that azacitidine was not only effective in therapy of CLL—a new finding—but it also kept a p53-deleted tumour under control. Today, the latter seems to be confirmed by some small studies; none of them, however, was done on CLL patients but on AML, MDS, and chronic myelomonocytic leukaemia patients [20–22]. While the OS after azacitidine therapy was negatively influenced by p53 mutations, overall response and complete remission did not differ significantly between patients with wild-type and mutated p53 [20, 21]. Also, sequential treatment with azacitidine followed by lenalidomide was equally effective in wild-type and mutated patients [22]. These as well as our observation that azacitidine is effective in both a p53-deleted (CLL) and a p53-non-deleted tumour (t-AML) support the idea that azacitidine acts independently of the functional status of p53.

After 12 months of azacitidine therapy, both the t-AML and CLL clones re-emerged. This synchronous control and re-appearance of both tumours suggest that azacitidine had suppressed both conditions by a similar mode of action.

Conclusions

Our data suggest that azacitidine is effective in the control of CLL and subsequent t-AML even in a patient with a rather poor prognosis. The simultaneous suppression and reappearance of the del(p53) CLL and the t-AML with wild-type p53 during azacitidine therapy suggests that azacitidine acts via a mechanism that is (i) similar in the different tumour types and (ii) independent of the p53 status. Based on these findings and assumptions, azacitidine might be worth testing as maintenance therapy for minimal residual disease in CLL.

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Informed consent
Oral informed consent was obtained from the patient described in this case report.

Conflict of interest
First author has received speaker honoraria and consultancy fees from Celgene, all other authors have no conflict of interest to declare.

References

1. Hallek M. Chronic lymphocytic leukemia: 2013 update on diagnosis, risk stratification and treatment. Am J Hematol. 2013;88(9):803–16.
2. Zenz T, Mertens D, Döhner H, Stilgenbauer S. Importance of genetics in chronic lymphocytic leukemia. Blood Rev. 2011;25(3):131–7.
3. Zenz T, Benner A, Döhner H, Stilgenbauer S. Chronic lymphocytic leukemia and treatment resistance in cancer: the role of the p53 pathway. Cell Cycle. 2008;7(24):3810–4.
4. Doubek M, Mayer J, Obrtlíková P, et al. Modern and conventional prognostic markers of chronic lymphocytic leukaemia in the everyday haematological practice. Eur J Haematol. 2011;87(2):130–7.
5. Byrd JC, Furman RR, Coutre SE, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. N Engl J Med. 2013;369(1):32–42.
6. Furman RR, Sharman JP, Coutre SE, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. N Engl J Med. 2014;370(11):997–1007.
7. Ricci F, Tedeschi A, Montillo M, Morra E. Therapy-related myeloid neoplasms in chronic lymphocytic leukemia and Waldenstrom's macroglobulinemia. Mediterr J Hematol Infect Dis. 2011;3:e2011031. doi:10.4084/MJHID.2011.031.
8. Stoddart A, McNerney ME, Bartom E, et al. Genetic pathways leading to therapy-related myeloid neoplasms. Mediterr J Hematol Infect Dis. 2011;3:e2011019. doi:10.4084/MJHID.2011.019.
9. Azacitidine (vidaza) Injection [EU prescribing information]. Switzerland: Celgene International; 2014.
10. Eltrombopag (revolade) Tablet [EU prescribing information]. United Kingdom: Glaxo Operations UK Ltd; 2010.
11. Colović M, Suvajdžić N, Janković G, et al. Therapy-related myelodysplastic syndrome and acute myeloid leukemia in patients with chronic lymphocytic leukemia treated with fludarabine and cyclophosphamide. Biomed Pharmacother. 2011;65(5):319–21.
12. Godley LA, Larson RA. Therapy-related myeloid leukemia. Semin Oncol. 2008;35(4):418–29.
13. Decitabine (dacogen) Powder [EU prescribing information]. Belgium: Janssen-Cilag Corporation; 2014.
14. Fianchi L, Criscuolo M, Lunghi M, et al. Outcome of therapy-related myeloid neoplasms treated with azacitidine. J Hematol Oncol. 2012;5(1):44.
15. Duong VH, Lancet JE, Alrawi E, et al. Outcome of azacitidine treatment in patients with therapy-related myeloid neoplasms with assessment of prognostic risk stratification models. Leuk Res. 2013;37(5):510–5.
16. Bally C, Thépot S, Quesnel B, et al. Azacitidine in the treatment of therapy related myelodysplastic syndrome and acute myeloid leukemia (tMDS/AML): a report on 54 patients by the Groupe Francophone Des Myelodysplasies (GFM). Leuk Res. 2013;37(6):637–40.
17. Malik A, Shoukier M, Garcia-Manero G, et al. Azacitidine in fludarabine-refractory chronic lymphocytic leukemia: a phase II study. Clin Lymphoma Myeloma Leuk. 2013;13(3):292–5.
18. Fenaux P, Gattermann N, Seymour JF, et al. Prolonged survival with improved tolerability in higher-risk myelodysplastic syndromes: azacitidine compared with low dose ara-C. Br J Haematol. 2010;149(2):244–9.
19. Fenaux P, Mufti GJ, Hellström-Lindberg E, et al. Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia. J Clin Oncol. 2010;28(4):562–9.
20. Bally C, Ades L, Renneville A, et al. Prognostic value of TP53 gene mutations in myelodysplastic syndromes and acute myeloid leukemia treated with azacitidine. Leuk Res. 2014;38(7):751–5.
21. Kulasekararaj AG, Smith AE, Mian SA, et al. TP53 mutations in myelodysplastic syndrome are strongly correlated with aberrations of chromosome 5, and correlate with adverse prognosis. Br J Haematol. 2013;160(5):660–72.
22. Platzbecker U, Braulke F, Kündgen A, et al. Sequential combination of azacitidine and lenalidomide in del(5q) higher-risk myelodysplastic syndromes or acute myeloid leukemia: a phase I study. Leukemia. 2013;27(6):1403–7.