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CASE REPORT

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Durable remissions with venetoclax monotherapy in secondary AML refractory to hypomethylating agents and high expression of BCL-2 and/or BIM

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Acute myeloid leukemia (AML) is a disease of the elderly population and survival remains poor after failure of hypomethylating agents (HMA). The BCL-2 inhibitor venetoclax demonstrated activity as monotherapy and in combination with chemotherapy or HMA in AML. In this case series, patients with secondary AML (sAML) not eligible for intensive chemotherapy and refractory to HMA were treated with venetoclax within a named patient program at our tertiary cancer center in Salzburg, Austria. Between April 2017 and September 2018, seven patients with sAML received venetoclax therapy. Two out of seven patients achieved a complete remission upon venetoclax initiation with a PFS of 505 days and 352 days and another patient achieved complete peripheral blood blast clearing within nine days after start of venetoclax. Among the venetoclax responders, primary refractory disease to prior HMA therapy was documented, 2 patients harbored IDH1/IDH2 mutations and one patient had an antecedent myeloproliferative neoplasm. High BCL-2 and/or BIM expression in myeloblasts was found in venetoclax responders and response was significantly associated with overall survival (responders: 364 days versus non-responders: 24 days, $P = 0.018$). Venetoclax monotherapy is safe and is able to induce durable responses in elderly patients with secondary AML after treatment failure with HMA.

KEYWORDS
azacitidine, BCL-2, BIM, hypomethylating agents, IDH1, IDH2, MCL-1, myeloproliferative neoplasm, secondary acute myeloid leukemia, venetoclax

1 | INTRODUCTION

Secondary acute myeloid leukemia (sAML) evolving from an antecedent hematological disorder and therapy-related sAML represent high-risk subsets of AML and are associated with poor clinical outcome.[1] The hypomethylating agents (HMA), azacitidine, and decitabine represent treatment options for elderly AML patients including sAML patients unfit for intensive chemotherapy.[2-5] Treatment options after HMA failure usually consist of BSC or low-dose cytarabine, and the prognosis remains limited with a median OS

Huemer and Melchardt equally contributed to this study.

TABLE 1 Patient characteristics and biomarker correlates of seven secondary AML patients treated with venetoclax

Patient	Age at AML diagnosis	Sex	Antecedent hematologic malignancy	Time to leukemic transformation (days)	Best response to HMA (IWG)	Cytogenetics	IDH1/2 mutation status	BCL-2 expression by IHC	MCL-1 expression by IHC	BIM expression by IHC
#24231	74	Female	MDS	483	TF	46 XX	IDH1 mutant	4	0	0
#8623	75	Female	MDS	1429	TF	46 XX	Wild-type	4	0.5	0.5
#6510	74	Male	MDS/MPN (CMML)	299	TF	46 XY	Wild-type	1	0	0
#25984	81	Female	MDS[a]	NA	PR	46 XX	Wild-type	1	0	1.0
#23769	65	Male	MDS[a]	NA	CRi	46 XY	Wild-type	1	0.5	1.0
#14501	73	Male	ET	1885	NA	46 XY	Wild-type	1	0	0.5
#17397	82	Male	PV	942	TF	NA	IDH2 mutant	1	1.0	1.5

BM, bone marrow; CMML, chronic myelomonocytic leukemia; CR, complete remission; CRi, complete remission with incomplete hematologic recovery; ET, essential thrombocythemia; HMA, hypomethylating agents; IDH, isocitrate dehydrogenase; IWG, International Working Group; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; NA, not available; OS, overall survival; PFS, progression-free survival; PR, partial remission; PV, polycythemia vera; sAML, secondary acute myeloid leukemia; TF, treatment failure; WBC, white blood count.
[a]Classified as sAML based on bone marrow aspirate/biopsy and/or peripheral blood smear.
[b]Response not evaluable by IWG criteria as no bone marrow re-evaluation was performed.

of 3.4 months.[6] Therefore, there is a high clinical demand for new therapeutic targets. BCL-2 mediates malignant cell survival by interfering with pro-apoptotic factors such as BAX, thereby preventing mitochondrial outer membrane permeabilization (MOMP) and finally preventing apoptosis.[7] Higher BCL-2 expression has prognostic impact and is associated with lower response rates to intensive chemotherapy and shorter survival in AML.[8,9] The selective oral BCL-2 inhibitor ABT-199 (venetoclax) has demonstrated promising responses in advanced-stage MDS, sAML,[10] and high-risk relapsed/refractory AML (including 54% with sAML) as monotherapy[11] as well as in combination with low-dose cytarabine[12] or with HMA[13,14] in elderly untreated AML patients unfit for intensive chemotherapy.

In this case series, we report the clinical outcome and biomarker correlates of seven elderly sAML patients receiving venetoclax after treatment failure with HMA.

2 | PATIENTS AND METHODS

Included patients were diagnosed with relapsed/refractory AML defined by the World Health Organization classification[15] and considered unfit for intensive induction chemotherapy. Venetoclax monotherapy was administered within a named patient program after failure of conventional therapies including HMA with a ramp-up dosing schedule and a target dose of 800 mg per day as previously reported.[11] All patients signed an informed consent for the off-label use of venetoclax, and all patients alive at the time point of data acquisition signed an informed consent to allow collection of personal data. Therapy response was evaluated by the revised International Working Group (IWG) criteria.[16] Primers for isocitrate dehydrogenase (IDH) 1 and 2 exon 4 analysis and PCR conditions were used as previously described.[17] Immunohistochemical staining was performed in myeloblasts based on pretreatment bone marrow aspirates/biopsies, which had been obtained during routine clinical care, using a Bond RXm system (Leica, Wetzlar) with primary antibodies against BCL-2 (M0887, DAKO, Agilent, Santa Clara, CA), BIM (ADI-AAP-330, Enzo Life Sciences, Farmingdale, NY), and MCL-1 (16225-1-AP, Rosemont, IL). Briefly, slides were deparaffinized using deparaffinization solution, pretreated with epitope retrieval solution 1 (corresponding to citrate buffer pH6) for 50 or 30 minutes, for MCL-1 and BCL-2, respectively, or epitope retrieval solution 2 (corresponding to EDTA buffer pH8) for 30 minutes for BIM. Antibody binding was detected with a polymer refine detection kit without postprimary reagent and visualized with DAB as a dark brown precipitate. Counterstaining was done with hematoxylin. As a positive control, healthy human tonsil tissue was used.

3 | RESULTS AND DISCUSSION

Between April 2017 and September 2018, seven patients with relapsed/refractory AML received venetoclax after treatment failure with HMA at our tertiary cancer center in Salzburg, Austria. At data cut-off (10/19/2018), all seven patients had discontinued venetoclax treatment due to progression and six patients had died. The patient baseline characteristics are shown in Table 1.

The median age at initial diagnosis of AML was 74 years and ranged between 65 and 82 years. All seven patients were diagnosed with secondary AML: four patients had an antecedent myelodysplastic syndrome (MDS), two patients developed leukemic

WBC at venetoclax start (G/L)	Best response to venetoclax (IWG)	Peripheral blast clearing during venetoclax	Survival status	PFS on prior therapy (days)	Non-hematologic venetoclax toxicity	Venetoclax dose modification	PFS on venetoclax (days)	OS from venetoclax initiation (days)
11.0	NA[b]	Day 9	Dead	222 (decitabine)	Diarrhea (III°)	Intermittent 200 mg dose (thrombocytopenia IV°)	70	126
0.7	CR	Day 21	Alive	110 (azacitidine)	None	None	505	549
9.0	TF	-	Dead	240 (azacitidine)	Fever (II°)	Temporary interruption	6	36
76.0	TF	-	Dead	325 (azacitidine)	None	None	6	15
2.0	TF	-	Dead	258 (azacitidine)	Unconjugated hyperbilirubinemia (II°)	None	18	24
269.0	TF	-	Dead	12 (azacitidine)	None	None	33	55
170.0	CR	Day 21	Dead	37 (azacitidine)	Unconjugated hyperbilirubinemia (III°)	Intermittent 200 mg dose	352	364

transformation based on chronic myeloproliferative neoplasms, and one patient suffered from antecedent chronic myelomonocytic leukemia. All patients were refractory to HMA at the start of venetoclax treatment with a median PFS of 222 days (range 12-325 days) on prior hypomethylating therapy. Median OS from venetoclax initiation was 55 days (range: 15-549 days, Figure 1A). One patient with antecedent MDS and one with antecedent myeloproliferative neoplasm achieved a complete remission with a PFS of 505 and 352 days, respectively. Despite the limited number of patients included in this retrospective analysis, exceptionally long response durations were observed with venetoclax monotherapy in pretreated sAML in comparison to previous studies.[11] A third patient derived antileukemic benefit from venetoclax and showed complete peripheral blood blast clearing by day 9 but did not meet response criteria defined by the IWG as no bone marrow evaluation was performed. Rapid disease progression (≤35 days upon venetoclax start) was documented in the four non-responders. High BCL-2 and/or BIM expression (Figure 1B) identified venetoclax responders, and response was associated with superior median OS (responders: 364 days vs non-responders: 24 days, $P = 0.018$, Figure 1C). High expression of BCL-2 may be a sign of so-called BCL-2 addiction, a concept describing a situation where cells expressing high levels of BH3-only proteins can only survive by overexpression of BCL-2. Such cells may be close to a threshold that can be effectively overcome by venetoclax.[18] On the other hand, a high expression of a pan-BCL-2-inhibitory BH3-only protein such as BIM may also indicate a therapeutic window of venetoclax, since its displacement may overcome MCL-1 dependent protection. Two of three venetoclax responders harbored IDH1 or IDH2 mutations, which are known to lower the mitochondrial threshold for initiation of apoptosis upon venetoclax therapy[19] and thereby increase response rates.[11] This may be associated with alterations in the BCL-2 family, but we were not able to discern a uniform pattern of BCL-2 family alterations. Interestingly, median OS from venetoclax initiation was significantly longer in patients with primary HMA refractory disease compared to HMA-responders (126 days vs 15 days; $P = 0.018$). In previous AML studies, patients with a WBC exceeding 25 G/L were excluded from venetoclax treatment.[11,14,20] Despite a baseline WBC of up to 269 G/L (range 0.7-269 G/L) at venetoclax initiation, we did not observe tumor lysis syndrome.

In summary, venetoclax monotherapy proved effective in elderly sAML patients after treatment failure with HMA, including a patient with antecedent myeloproliferative neoplasm. Responses were clinically apparent within 4-6 weeks, favoring a short trial of venetoclax in patients without standard options. High baseline BCL-2 and/or BIM expression may identify responders to venetoclax treatment. These findings should be validated in future clinical trials.

CONFLICTS OF INTEREST

Florian Huemer: none; Thomas Melchardt: honoraria (Abbvie); Bettina Jansko: none; Adam Wahida: none; Stefanie Jilg: none; Philipp J. Jost: research grant (Abbvie); Eckhard Klieser: none; Katja Steiger: none; Teresa Magnes: none, Lisa Pleyer: consulting (Celgene), honoraria (Celgene), travel grants (Celgene); Sigrun Greil-Ressler: none; Christof Rass: none; Richard Greil: honoraria (Celgene), consulting (Celgene, Abbvie), research grant (Celgene), travel grants (Janssen); Alexander Egle: honoraria (Abbvie), consulting (Abbvie, Celgene, Janssen), travel grants (Abbvie).

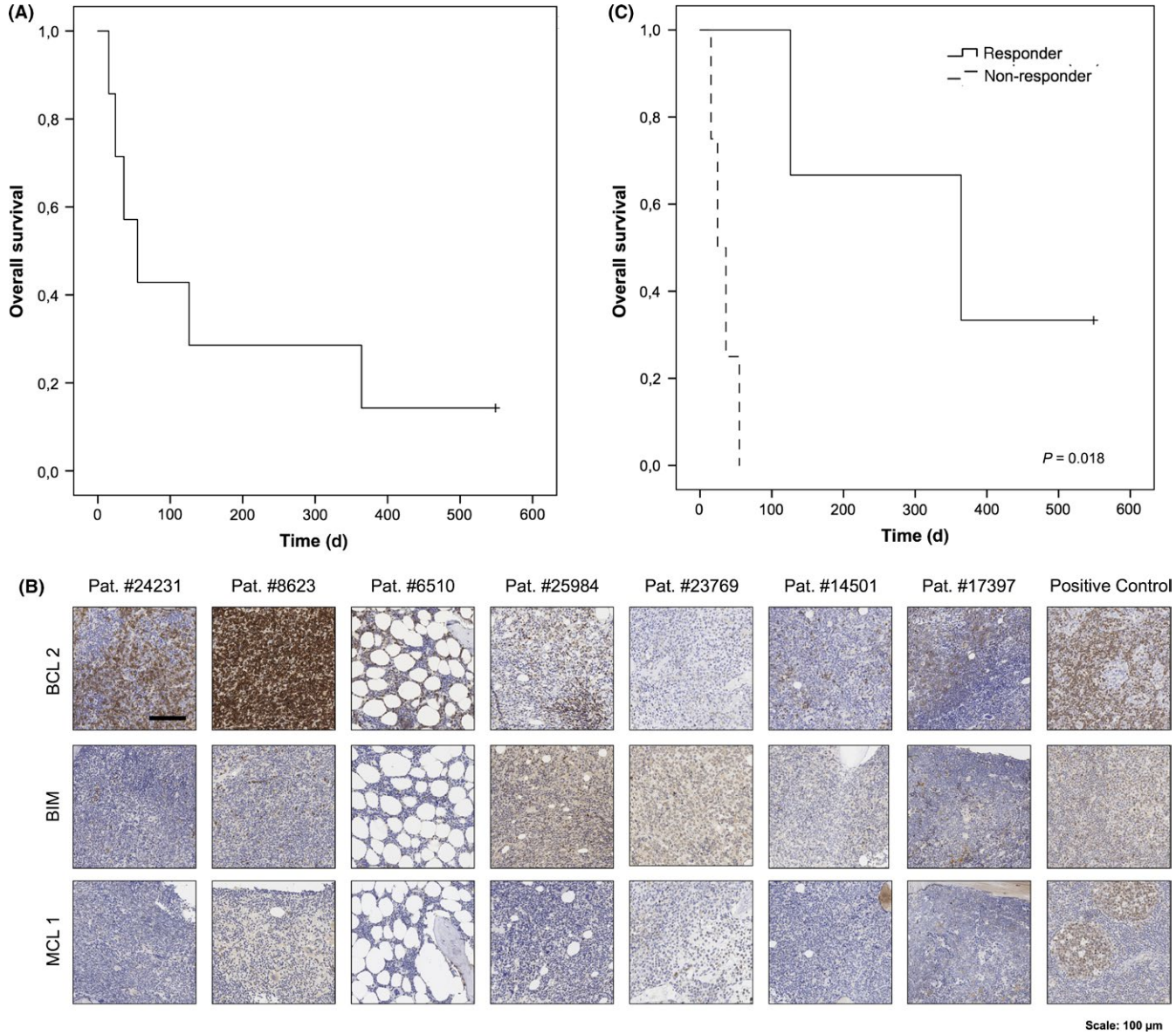

FIGURE 1 Overall Survival of Seven Secondary AML Patients Refractory to Hypomethylating Agents from Venetoclax Initiation (A), Immunohistochemistry for BCL-2, BIM, and MCL-1 on Myeloblasts (B), and Overall Survival From Venetoclax Initiation Based on Venetoclax Therapy Response (C). A, The tick marks on the curves represent censored patients. B, BCL-2, BIM, and MCL-1 expression on myeloblasts based on pretreatment bone marrow biopsies/aspirates. C, Responders included patient #8623 (CR), #17397 (CR), and #24231 (rapid peripheral blast clearing). The tick marks on the curves represent censored patients

AUTHOR CONTRIBUTIONS

FH collected and interpreted the data and wrote the manuscript. Th.M. collected and interpreted the data and wrote the manuscript. BJ performed the IDH mutation analyses. AW performed the immunohistochemical analysis and interpretation. SJ performed the immunohistochemical analysis and interpretation. PJ interpreted the data and edited the manuscript. KS provided technical assistance and advice for staining of primary human samples via immunohistochemistry and interpreted the data. EK performed the immunohistochemical analyses of the bone marrow biopsies. TM interpreted the data and edited the manuscript. LP interpreted the data and edited the manuscript. S.G-R interpreted the data and edited the manuscript. CR interpreted the data and edited the manuscript. RG interpreted the data, edited the manuscript and supervised this work. AE conceived the report on this case series including the retrospective molecular analyses, interpreted the data, edited the manuscript and supervised this work. All authors have read and approved the final manuscript. All co-authors provided continuous intellectual guidance, repeatedly reviewed the manuscript, and gave the final approval for submission.

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REFERENCES

1. Boddu P, Kantarjian HM, Garcia-Manero G, et al. Treated secondary acute myeloid leukemia: a distinct high-risk subset of AML with adverse prognosis. *Blood Adv*. 2017;1(17):1312-1323.
2. Dombret H, Seymour JF, Butrym A, et al. International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts. *Blood*. 2015;126(3):291-299.
3. Kantarjian HM, Thomas XG, Dmoszynska A, et al. Multicenter, randomized, open-label, phase III trial of decitabine versus patient choice, with physician advice, of either supportive care or low-dose cytarabine for the treatment of older patients with newly diagnosed acute myeloid leukemia. *J Clin Oncol*. 2012;30(21):2670-2677.
4. Falantes J, Pleyer L, Thepot S, et al. Real life experience with frontline azacitidine in a large series of older adults with acute myeloid leukemia stratified by MRC/LRF score: results from the expanded international E-ALMA series (E-ALMA+). *Leuk Lymphoma*. 2018;59(5):1113-1120.
5. Dumas PY, Bertoli S, Berard E, et al. Azacitidine or intensive chemotherapy for older patients with secondary or therapy-related acute myeloid leukemia. *Oncotarget*. 2017;8(45):79126-79136.
6. Prebet T, Gore SD, Thepot S, et al. Outcome of acute myeloid leukaemia following myelodysplastic syndrome after azacitidine treatment failure. *Br J Haematol*. 2012;157(6):764-766.
7. Chen L, Willis SN, Wei A, et al. Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. *Mol Cell*. 2005;17(3):393-403.
8. Campos L, Rouault JP, Sabido O, et al. High expression of bcl-2 protein in acute myeloid leukemia cells is associated with poor response to chemotherapy. *Blood*. 1993;81(11):3091-3096.
9. Lauria F, Raspadori D, Rondelli D, et al. High bcl-2 expression in acute myeloid leukemia cells correlates with CD34 positivity and complete remission rate. *Leukemia*. 1997;11(12):2075-2078.
10. Jilg S, Reidel V, Muller-Thomas C, et al. Blockade of BCL-2 proteins efficiently induces apoptosis in progenitor cells of high-risk myelodysplastic syndromes patients. *Leukemia*. 2016;30(1):112-123.
11. Konopleva M, Pollyea DA, Potluri J, et al. Efficacy and biological correlates of response in a phase II study of venetoclax monotherapy in patients with acute myelogenous leukemia. *Cancer Discov*. 2016;6(10):1106-1117.
12. Wei A, Strickland SA, Roboz GJ, et al. Phase 1/2 study of venetoclax with low-dose cytarabine in treatment-naive, elderly patients with acute myeloid leukemia unfit for intensive chemotherapy: 1-year outcomes. American Society of Hematology 59th Annual Meeting. 2017.
13. DiNardo CD, Pollyea DA, Jonas BA, et al. Updated safety and efficacy of venetoclax with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. American Society of Hematology 59th Annual Meeting. 2017.
14. DiNardo CD, Pratz K, Pullarkat V, et al. Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. *Blood*. 2018;133:7-17.
15. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2405.
16. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the international working group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. *J Clin Oncol*. 2003;21(24):4642-4649.
17. Willander K, Falk IJ, Chaireti R, et al. Mutations in the isocitrate dehydrogenase 2 gene and IDH1 SNP 105C > T have a prognostic value in acute myeloid leukemia. *Biomark Res*. 2014;2:18.
18. Certo M, Del Gaizo MV, Nishino M, et al. Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. *Cancer Cell*. 2006;9(5):351-365.
19. Chan SM, Thomas D, Corces-Zimmerman MR, et al. Isocitrate dehydrogenase 1 and 2 mutations induce BCL-2 dependence in acute myeloid leukemia. *Nat Med*. 2015;21(2):178-184.
20. DiNardo CD, Pratz KW, Letai A, et al. Safety and preliminary efficacy of venetoclax with decitabine or azacitidine in elderly patients with previously untreated acute myeloid leukaemia: a non-randomised, open-label, phase 1b study. *Lancet Oncol*. 2018;19(2):216-228.

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