blood advances

TO THE EDITOR:

Gastrointestinal toxicity of gemtuzumab ozogamicin: real-life data from the AMLCG, SAL, and CELL study groups

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The CD33-directed antibody-drug-conjugate "gemtuzumab ozogamicin" (GO) in combination with the 7+3 standard regimen is currently approved for the treatment of $CD33^+$ acute myeloid leukemia (AML). Although the pivotal ALFA-0701 study did not show a significantly increased toxicity using 7+3 + GO compared with 7+3,[1] a slightly higher rate of severe gastrointestinal (GI) toxicity was reported (16% vs 10%), and additional evidence of GO-associated GI toxicity was published.[2,3] We observed several cases of severe GI toxicity and therefore analyzed patients treated with GO in a real-world setting.

Our findings are based on a single-center analysis of patients treated at the University Hospital Munich and a multicenter analysis from 32 hospitals of the German SAL (Study Alliance Leukemia), the AMLCG (AML Cooperative Group), the CELL (Czech Leukemia Study Group), and other university hospitals of Germany. For further information about the methods used in our analyses, refer to supplemental Methods.

A total of 115 patients were included in the single-center analysis (GO, n = 25; control [C], n = 90). Showing a comparable gender distribution (GO vs C, 52% vs 51% female) and median age (GO vs C, 57 years [range, 28-73] vs 58 years [range, 19-78]), the risk classification differed as expected (GO vs C, 72% vs 33% favorable genotypes). The median pretherapeutic white blood cell count was significantly different (GO vs C, 17.4×10^9/L vs 28.9×10^9/L; $P = .001$). All the 25 patients of the GO cohort were treated with only 1 induction cycle, whereas 12% of the C cohort received a second cycle. Further patient and treatment details are shown in supplemental Table 1. Although most of the assessed toxicity occurred equally frequent, the rates of grade 3 to 4 enteritis/colitis (median onset after 12.5 days) were numerically higher in the GO cohort (GO vs C, 24% [6/25 patients] vs 10% [9/90 patients]; $P = .09$; Figure 1A). No cases of grade 5 enteritis/colitis were documented. Several of these patients were confirmed to have neutropenic enterocolitis, which was again more frequent in the GO group (GO vs C, 20% [5/25] vs 8% [7/90]; $P = .13$; Figure 1B). Same is true when looking at patients suffering from severe enteritis/colitis that led to life-threatening complications, including intensive care unit (ICU) admission or surgical intervention: there was only 1 patient (patient ID, C4; ICU admission) of 90

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Original data are available on request from the corresponding author, Ricarda Knabe (ricarda.knabe@med.uni-muenchen.de).

The full-text version of this article contains a data supplement.

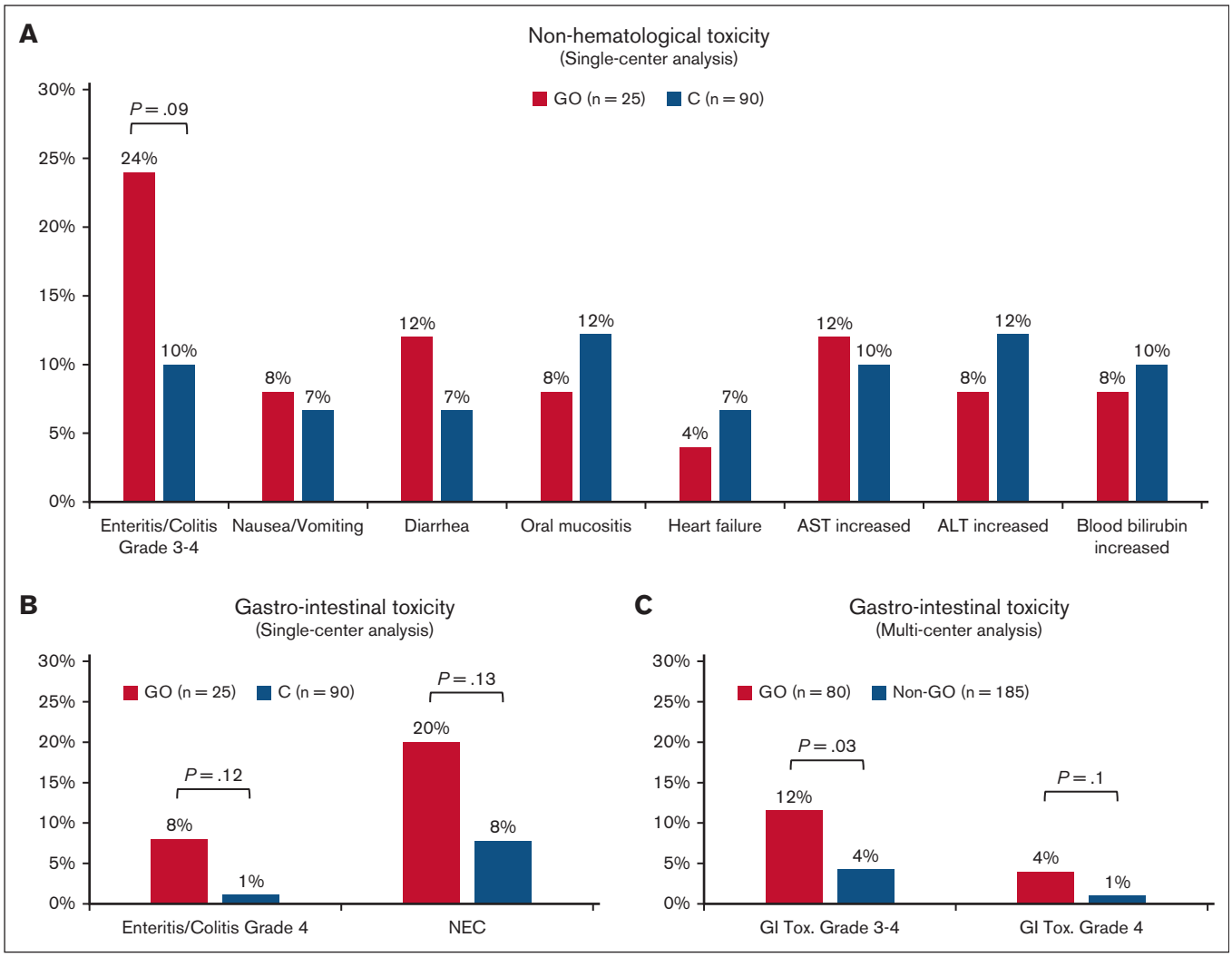

Figure 1. Nonhematological toxicity. (A) Bar plot of relative frequencies of nonhematological toxicity grade 3 to 4; enteritis/colitis (using Common Terminology Criteria for Adverse Events [CTCAE] V 3.0 criteria; $P = .09$ [2-sided Fisher exact test, α level, .05]); and relative frequencies of nonhematological grade 3 to 4 toxicity; other categories (using CTCAE V 5.0 criteria) of patients being treated with (red column) or without (blue column) an induction regimen including GO in the single-center analysis. (B) Bar plot of relative frequencies of grade 4 enteritis/colitis (using CTCAE V 5.0; $P = .12$ [2-sided Fisher exact test, α level, .05]); and relative frequencies of neutropenic enterocolitis[4] ($P = .13$ [2-sided Fisher exact test, α level, .05]) of patients treated with GO (red column) or without GO (blue column) as part of the induction therapy in the single-center analysis. (C) Bar plot of relative frequencies of GI toxicity grade ≥3 (including diarrhea, nausea/vomiting, enteritis/colitis, and stomach pain using CTCAE V5; $P = .03$ [χ^2 test]) and relative frequencies of GI toxicity grade 4 ($P = .1$ [Fisher exact test]) of patients treated with GO (red column) or without GO (blue column) as part of the induction therapy in the multicenter analysis. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GI Tox., gastro intestinal toxicity; NEC, neutropenic enterocolitis.

patients in the C group, compared with 2 patients of 25 patients in the GO group (GO vs C, 8% vs 1%; $P = .12$; Figure 1B). Figure 2A-B shows the pathological findings in abdominal computed tomography imaging of these 2 GO patients in the single-center cohort (patient G1, prolonged ICU admission (>40 days); patient G2, emergency hemicolectomy). None of the patients with grade 3 to 4 enteritis/colitis had any documented preexisting GI diseases (supplemental Table 2).

We next evaluated CD33 expression and apoptosis in 10 healthy colon specimens and in the ileocecal resection specimen of patient G2 (Figure 2C-H). CD33 was expressed in immune and stromal cells of the lamina propria but not in epithelial cells. The number of positive cells per high-power field (HPF) in the patient was within the range of a normal colon (patients, n = 15; normal colon, n = 17 ± 3). Apoptosis was increased in the patient's colon (patient, n = 6/10 HPF; vs normal colon, n = 1/10 HPF), supporting the diagnosis of neutropenic enterocolitis.

The multicenter analysis included 265 patients (GO group, n = 80 patients; non-GO group, n = 185 patients). The median age of these patients with core binding factor (CBF)-AML was 47 years (range, 19-75) for the GO-group and 49 years (range, 18-81) for the non-GO group. Twenty-three Ludwig-Maximilians-Universität (LMU) patients were excluded because some had already been evaluated for the single-center analysis. The rates of grade 3 to 4 GI toxicity (median onset after 12 days) were significantly higher in the GO group than in the non-GO group (GO vs non-GO, 12%

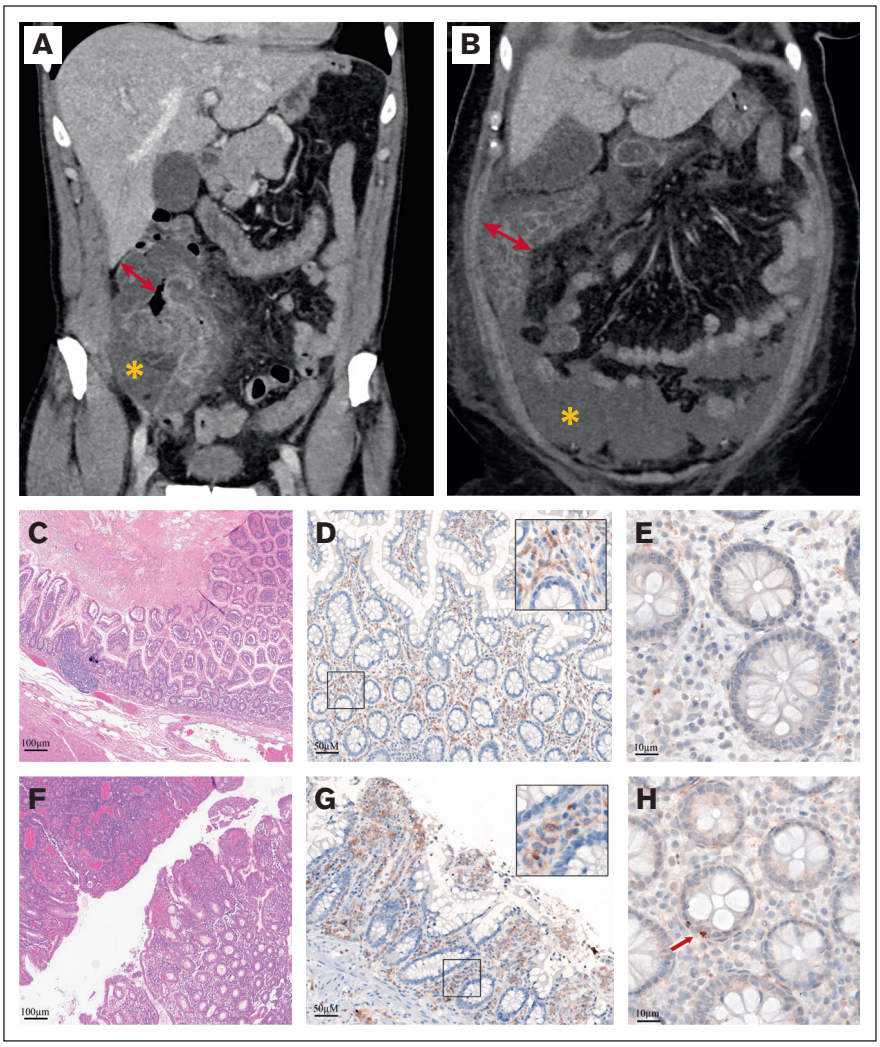

Figure 2. CT imaging and histological examination of bowels affected by neutropenic enterocolitis. (A-B) Abdominal computed tomography (CT) imaging of patients (G2, left; G1, right) being treated with a GO-containing therapy regimen with grade 4 enteritis/colitis. Clinical and CT-based findings confirmed them to be cases of neutropenic enterocolits; red arrow indicates colonic wall thickening; yellow star, ascites and peritoneal contrast enhancement as a sign of peritoneal irritation. Immunohistochemistry was performed on 6-μm sections of formalin-fixed, paraffin-embedded tissue. After antigen retrieval, slides were incubated with antibodies against CD33 (PWS44; Leica) or cleaved caspase-3 (5A1E; 1:200; Cell Signaling Technology). The signal was visualized using the ImmPress anti-rabbit immunoglobulin G polymer kit (Vector Laboratories) according to the manufacturer's instructions. The total number of positive cells was quantified in 10 HPFs. H&E(hematoxylin and eosin) staining of colon (normal [C] and colitis [F]). CD33 stains stromal cells in normal (D) and inflamed colon (G), whereas no staining is observed in epithelial cells. Immunohistochemistry for cleaved caspase 3 (normal [E] and colitis [H]) highlights apoptotic epithelial cells (indicated by red arrow). Panels F-H are specimens of the ileocecal resection of a patient being treated with 3+7 + GO (G2).

vs 4%; $P = .03$; Figure 1B). Consistent with the findings in our single-center analysis, cases of severe GI complications (grade 4) were seen more often in the GO group than in the non-GO group (GO vs non-GO, 4% vs 1%; $P = .1$; Figure 1C).

Because we saw a numerically higher toxicity rate in the single-center analysis than in the multicenter analysis (24% vs 12%), we performed a logistic regression to detect whether the impact of GO/non-GO on grade 3 to 4 GI toxicity differed between both cohorts. The odds ratio of the interaction term (0.97; 95% confidence interval, 0.21-4.57; $P = .97$) does not indicate any difference in the effect of GO on grade 3 to 4 GI toxicity across both cohorts (single-center vs multicenter analysis; supplemental Figure 2), with numerically similar odds ratios of 2.8 (multicenter) and 2.9 (single-center) for grade 3 to 4 GI toxicity.

We describe clinically relevant and statistically significant higher GI toxicity in GO-treated patients. A similar trend was seen in the pivotal ALFA-0701 trial (grade 3-4 GI adverse events for GO vs C, 16% vs 10%; $P = .21$). What remains unclear is why the rate of GI toxicity in our single-center analysis tends to be higher than ALFA-0701. The assessed baseline criteria (median age, risk classification, therapy regimen, and pretherapeutic white blood cell) did not differ in a way that would explain this observation. Because a hospital with higher rates of complications is more likely to initiate an analysis such as this, the numerically higher incidence in our single-center analysis than the multicenter analysis might be due to selection bias and random variability. Furthermore, this finding might be explained by the more detailed analysis of patients included in the single-center analysis in which data could be directly drawn from the digital documentation system of the LMU Munich. The ALFA-0701 trial unfortunately does not provide any further details about the criteria applied for the evaluation of GI adverse events.

At first sight, the higher rate of GI toxicity seen in the single-center analysis than the multicenter analysis might have been confounded by the higher median age of the LMU cohort. However, the relative effects of GO on severe GI toxicity were very similar in both our cohorts, as revealed by logistic regression incorporating an interaction between cohorts and treatment, with and without age adjustment.

A higher rate of GI toxicity might also be due to the different settings of our real-life analysis and the clinical ALFA0701 trial. To our

knowledge, no other data on GI toxicity in a real-world cohort of patients receiving a GO-containing induction therapy have been published so far.

The underlying pathophysiology of GO-associated GI toxicity is another issue worthy of further investigations. According to literature, CD33 expression in the bowel is quite low (supplemental Figure 1).[5] We showed here that its expression is restricted to immune and stromal cells of the bowel.

Furthermore, the dosage of GO is a topic to be discussed. Most of the patients in our analysis received 3 doses of GO according to the ALFA0701 trial. Other trials such as AML-18 showed a response benefit of a fractioned schedule (2 doses) vs a single dose of GO.[6] In contrast to our analysis, in this trial, no grade 4 GI toxicity was reported. Therefore, regimens with 2 doses of GO might be sufficient and less toxic. Further studies to clarify this issue are warranted.

In conclusion, for the administration of GO in clinical routine, patients at higher risk of developing GI toxicity (eg, preexisting inflammatory bowel disease and higher age, etc) should be carefully assessed for the expected risk-benefit ratio of GO treatment. Reduction of GO dose might be a suitable approach to reduce toxic effects in this subset of patients.

Contribution: R.K. designed research, performed research, collected data, analyzed and interpreted data, performed statistical analysis, and wrote the manuscript; P.M., C.S., M.H., J.M.U., B.W., J.M., C.K., S.W.K., M.K., S.K., B.S., and F.M. involved in patient care, collected data, provided critical feedback, and revised the manuscript; M.S. and V.B. involved in patient care, provided critical feedback, and revised the manuscript; W.G.K. and M.R. involved in patient care, collected data, analyzed and interpreted data, provided critical feedback, and revised the manuscript; M.v.B.-B. involved in patient care, provided critical feedback, and revised the manuscript; K.G. and E.H. analyzed and interpreted data, performed statistical analysis, and wrote the manuscript; T.H. involved in patient care, provided critical feedback, and revised the manuscript; C.R. involved in patient care, provided critical feedback, and wrote the manuscript; and K.S. involved in patient care, designed research, performed research, collected data, analyzed and interpreted data, provided critical feedback, and wrote the manuscript.

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