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ORIGINAL ARTICLE – CANCER RESEARCH

Clinical and biological characteristics of myeloma patients influence response to elotuzumab combination therapy

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Abstract

Based on ELOQUENT-2, combination therapy with the monoclonal antibody elotuzumab was approved for relapsed/refractory multiple myeloma in the US and Europe. However, outside clinical trials, the optimal integration of elotuzumab into the sequence of treatment lines remains to be determined. Therefore, we analyzed safety and efficacy of elotuzumab/immunomodulatory drug combinations in a real-life cohort of 33 patients from our institution. The most frequent grade 3/4 adverse event was lymphopenia which did not increase the incidence of viral reactivations. After a median of four prior treatment lines, an overall response rate of 60% and a median progression-free survival (PFS) of 8 months were observed. The presence of cytogenetic high-risk status had no impact on PFS while low disease burden and high numbers of natural killer (NK)-cells at treatment initiation were associated with longer PFS. We observed an extramedullary relapse in three patients, associated with reduced expression of the elotuzumab target antigen SLAMF7 on extramedullary myeloma cells in one patient. Thus, biomarkers like disease burden, NK-cell count and SLAMF7 expression on myeloma cells may help to define myeloma patients with high likelihood to respond to elotuzumab treatment. Prospective trials investigating these biomarkers in larger patient cohorts are highly warranted.

Keywords Multiple myeloma · SLAMF7 · Immunotherapy · Natural killer cells · Extramedullary disease

Introduction

Never before in the history of myeloma treatment, has the FDA approved more novel antimyeloma agents within 1 year than in 2015 (Dimopoulos et al. 2016b, 2017a; Lonial et al. 2015; Moreau et al. 2016; Palumbo et al. 2016; Richardson et al. 2015; San-Miguel et al. 2014). With subsequent EMA approvals, two monoclonal antibodies (mAbs) have now entered the field of myeloma treatment in the US and Europe. While the anti-CD38 mAb daratumumab has led to enthusiasm due to its single-agent efficacy (Lonial et al. 2016; van de Donk et al. 2016), elotuzumab has demonstrated efficacy in combination therapy only (Chim et al. 2017; Hofmeister and Lonial 2016).

The elotuzumab target signaling lymphocytic activation molecule F7 (SLAMF7; CS1, CD319) is highly and uniformly expressed on myeloma cells (Hsi et al. 2008), but it can also be found on a subset of normal lymphocytes like natural killer (NK) cells and T cells (Boles et al. 2001; Gogishvili et al. 2017). Besides induction of antibody-dependent cellular cytotoxicity (ADCC), elotuzumab exerts antimyeloma effects by activation of NK cells via SLAMF7 ligation (Collins et al. 2013). However, elotuzumab monotherapy has also resulted in lymphopenia which was attributed to a reduction of NK cells (Zonder et al. 2012). To what extent this interferes with the NK-cell-propagating effects of immunomodulatory drugs (IMiDs) (Danhof et al. 2017; Davies et al. 2001) has not been investigated yet.

The function of SLAMF7 on myeloma cells was associated with homing and adherence of plasma cells to the bone marrow niche (Tai et al. 2008). In preclinical models,

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elotuzumab reduced the adherence of myeloma cells to the bone marrow stromal cells (Tai et al. 2008), thus depriving the malignant cells from the protective effects of the bone marrow microenvironment. However, it has not been investigated whether this mechanism can increase the release of myeloma cells from the bone marrow niche and thus provoke extramedullary relapses.

The clinically most relevant question is the optimal integration of elotuzumab into the sequence of different treatment lines. Data from the registration trial ELOQUENT-2 suggested that patients with a low number of prior lines of therapy benefit most from elotuzumab treatment (Dimopoulos et al. 2017a). However, to date, phase III efficacy data with elotuzumab on patients relapsing after first-line treatment with the standard antimyeloma agents—including lenalidomide—are limited (Laubach et al. 2017). Given the wide range of alternative treatment options (Dimopoulos et al. 2016a, b, 2017b; Palumbo et al. 2016; San-Miguel et al. 2014; San Miguel et al. 2013), biomarkers which help to define the patients most likely to benefit from elotuzumab therapy are highly warranted. We have thus retrospectively analyzed a "real life" cohort of 33 patients with relapsed/refractory myeloma. All patients received a triplet combination therapy of elotuzumab, an IMiD and dexamethasone at our institution. Subgroup analyses according to biological and clinical characteristics identified patient cohorts with above-average response to the treatment. Our observations can potentially pave the way towards a more specific use of elotuzumab by providing a rationale for biomarker-guided clinical decision-making in the future.

Patients and materials and methods

Patient selection

Clinical records were reviewed for all myeloma patients with clinical or serological progression who were treated with a combination therapy of elotuzumab, an IMiD and dexamethasone at our institution since December 2015 (33 patients). Assessment of toxicity was performed for all patients and response was assessed for those who had completed at least one treatment cycle (30 patients). Cut-off date for the collection of follow-up data was 24 October 2017. The retrospective analysis of clinical data and the analysis of blood and bone marrow samples from myeloma patients and healthy donors were approved by the ethical committee of the University of Wuerzburg, Medical Faculty (reference numbers: 275/13, 60/18 and 61/18; date of issue: 19/11/2013, updated on 27/02/2018).

Assessment of response and toxicities

Evaluation of response was performed according to the International Myeloma Working Group Response Criteria (Kumar et al. 2016). Progression-free survival (PFS) was defined as time from first application of elotuzumab until confirmation of disease progression (PD) or death from any cause. Overall survival (OS) was measured from first application of elotuzumab until death from any cause and the remaining patients were censored at the last date of follow-up. Adverse events (AEs) were classified and graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE v4.0). For each patient, the highest documented grade of every type of adverse event was noted. In addition, the occurrence of hematologic AEs was illustrated in aligned dot plots showing laboratory results before and nadir values during treatment.

Blood samples and cell phenotyping

EDTA-anticoagulated peripheral venous blood samples were obtained from healthy donors ($n=8$) and a subgroup of myeloma patients from the current cohort prior to elotuzumab-based treatment ($n=10$). All individuals had provided written informed consent according to the Declaration of Helsinki. The study was approved by the ethical committee of the University of Wuerzburg, Medical Faculty. Absolute lymphocyte counts in blood samples were evaluated by local clinical routine laboratories.

Lymphocyte subset composition and cell surface expression of SLAMF7 by $CD4^+$ T cells, $CD8^+$ T cells, $CD3^-CD56^+$ natural killer (NK) cells, $CD3^+CD56^+$ NKT cells, $CD3^-CD19^+$ B cells, $CD3^+$ $CD4^+CD25^+Foxp3^+$ regulatory T cells and $CD3^+\gamma/\delta TCR^+$ T cells were determined in whole blood lysates using multi-parameter flow cytometry. The gating strategy is depicted in Supplementary Fig. 1. The cells were stained with the following conjugated monoclonal antibodies: Foxp3 APC (eBioscience, San Diego, CA), CD3 PerCP (all BD Biosciences, San Jose, CA), SLAMF7 PE, CD4 Pacific Blue, γδTCR APC, CD19 PECy7, CD56 Brilliant Violet 421, IgG1 APCκ, IgG2bκ PE (all BioLegend, San Diego, CA), and CD4 PEVio770, CD8 FITC (Miltenyi Biotec, Bergisch Gladbach, Germany). Flow cytometry was performed using a FACSCanto II cytometer (BD Biosciences, San Jose, CA) and data were analyzed using FlowJo software version 9 (Tree Star Inc., Ashland, OR).

Statistical analyses

Descriptive data are presented as absolute counts and percentages for categorical variables and as medians and ranges for continuous variables. Statistical significance was evaluated using two-tailed Student's *t* test for normally distributed data and significance levels of $p<0.05$ (*), $p<0.01$ (**), and $p<0.001$ (***) were defined. Time-to-event data (PFS, OS) were analyzed using the Kaplan–Meier method, and group comparisons were performed using log-rank test. Statistical computations were performed and artwork was created using GraphPad Prism version 5 (La Jolla, CA).

Results

Patient characteristics and treatment plan

From December 2015 to October 2017, 33 myeloma patients received elotuzumab-based treatment at our tertiary care center (Table 1). Treatment schedule consisted of two initial 4-week cycles with weekly elotuzumab administration

Table 1 Clinical patient characteristics at start of elotuzumab treatment

	All (33)	Len (19)	Pom (14)
Median age (range)—yr	65 (40–82)	65 (40–78)	66 (50–82)
Male—no. (%)	20 (61)	9 (47)	11 (79)
Salmon and Durie[a] at diagnosis—no. (%)			
I	4 (12)	3 (16)	1 (7)
II	9 (27)	5 (26)	4 (29)
IIIA	17 (52)	9 (47)	8 (57)
IIIB	3 (9)	2 (11)	1 (7)
Myeloma subtype—no. (%)			
IgG	21 (64)	12 (63)	9 (64)
IgA	7 (21)	4 (21)	3 (21)
IgD	1 (3)	1 (5)	0 (0)
LC	4 (12)	2 (11)	2 (14)
Cytogenetic profile[b]—no. (%)			
High risk	10 (30)	6 (32)	4 (29)
Standard risk	16 (48)	8 (42)	8 (57)
ND	7 (21)	5 (26)	2 (14)
Time from diagnosis (range)—months	68 (15–228)	67 (15–228)	70 (23–204)
Previous therapy regimens			
Median no. (range)	4 (1–11)	3 (1–8)	4 (2–11)
Previous therapies—no. (%)			
Hematopoietic stem-cell transplantation	29 (88)	16 (84)	13 (93)
1×	5 (15)	4 (21)	1 (7)
2×	15 (45)	8 (42)	7 (50)
3×	9 (27)	4 (21)	5 (36)
Immunomodulatory drug	32 (97)	18 (95)	14 (100)
Thalidomide	7 (21)	5 (26)	2 (14)
Lenalidomide	31 (94)	17 (89)	14 (100)
Pomalidomide	14 (42)	5 (26)	9 (64)
Refractory to any IMiD	12 (36)	4 (21)	8 (57)
Proteasome inhibitor	33 (100)	19 (100)	14 (100)
Bortezomib	33 (100)	19 (100)	14 (100)
Carfilzomib	6 (18)	2 (11)	4 (29)
Refractory to any proteasome inhibitor	13 (39)	7 (37)	6 (43)
Polychemotherapy[c]	10 (30)	4 (21)	6 (43)
Experimental treatment/phase I trial	8 (24)	5 (26)	3 (21)

Len lenalidomide, *Pom* pomalidomide, *yr* years, *LC* light chain, *ND* not done

[a]A clinical staging system for multiple myeloma (Durie and Salmon 1975)

[b]Cytogenetic analysis: high-risk cytogenetic profile refers to adverse FISH including IgH translocations (t(4;14) or t(14;16) or t(14;20)), 17p13 del and/or 1q21 gain (Chng et al. 2014)

[c]Such as VTD-PACE, DCEP, DexaBEAM

at 10 mg/kg bodyweight followed by 4-week cycles with elotuzumab dosing on days 1 and 15 (Supplementary Table 1). Treatment was scheduled as continuous therapy according to its approval status. Besides low-dose dexamethasone, the triplet combination included lenalidomide in 19 patients (58%) and pomalidomide in 14 patients (42%; Supplementary Table 1). Medical prophylaxis of venous thromboembolism, reactivation of herpes zoster and *Pneumocystis jirovecii* pneumonia was recommended to patients at risk. All patients had relapsed/refractory myeloma with a median of 4 (range 1–11) prior lines of therapy and elotuzumab treatment was initiated at a median of 68 (range 15–228) months from diagnosis. Most patients had previously undergone autologous hematopoietic stem cell transplantation (HSCT; 88%) and had received treatment with an immunomodulatory drug (IMiD; 97%) and a proteasome inhibitor (100%). Seven (21%) and five patients (15%) were refractory to one or two IMiDs, respectively (*Supplementary Table 2*). Four (12%) patients had developed their refractoriness in the previous treatment line, whereas eight (24%) patients had intermittently received up to four different lines of myeloma treatment and were now re-challenged with an IMiD. For the elotuzumab combination regimen, the IMiD was individually selected by the treating physician according to patients' intolerances, previous treatment and clinical judgement. Most baseline characteristics were balanced between the lenalidomide and the pomalidomide cohorts. However, patients in the pomalidomide cohort were more heavily pretreated with two or more HSCTs (86%) and carfilzomib (29%) and more than half of the patients (57%) were refractory to an IMiD.

Treatment discontinuation before completion of the first cycle occurred in three patients (9%) and was due to patient's (two patients) or physician's (one patient) choice. These patients were eligible for safety, but not efficacy analyses. At database lock, eight patients were still on treatment while the remaining 22 patients had discontinued treatment after at least one full cycle, mostly due to disease progression (18 patients). Further reasons for discontinuation included patient's choice (two patients), the occurrence of a symptomatic deep venous thrombosis despite adequate prophylaxis with low-molecular weight heparin and death due to other cause, respectively (one patient each).

Evaluation of response

For evaluation of response, only patients who had completed at least one treatment cycle were included (30 patients, 91%). Those patients received a median of 5 (range 1–23) cycles and median PFS was 8 (range 1–21) months (Fig. 1a). The overall response rate was 60% with two (7%) patients achieving very good partial response (VGPR) and 16 (53%) achieving partial response (PR). Seven (23%) patients maintained stable disease (SD). Median time to response was 8 (range 3–30) weeks and median duration of response was 17 (1–87) weeks.

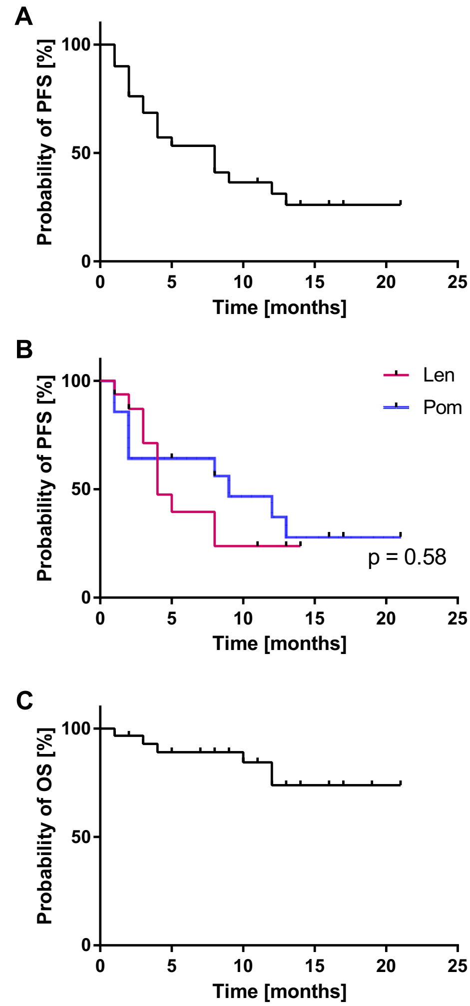

Fig. 1 Progression-free and overall survival. **a, b** Kaplan–Meier curve of progression-free survival (PFS) for all patients who completed at least one treatment cycle (**a** median PFS: 8 months) and separate curves for the subgroups of patients treated with lenalidomide (Len; red; median PFS: 4 months) or pomalidomide (Pom; blue; median PFS: 9 months), respectively (**b**). **c** Kaplan–Meier curve of overall survival (OS) for all patients who completed at least one treatment cycle (median OS: not reached). *PFS* progression-free survival, *Len* lenalidomide, *Pom* pomalidomide, *OS* overall survival

Patients with lenalidomide combination received a median of 4 (1–14) cycles and had a PFS of 4 months, whereas patients with pomalidomide combination received a median of 8 (1–23) cycles and had a PFS of 9 months. The difference was not statistically significant ($p = 0.58$; Fig. 1b). At a median follow-up of 12 (range 0–21) months, the median overall survival has not been reached in both cohorts (Fig. 1c).

Association of patient or disease characteristics with response to treatment

Subgroup analyses from ELOQUENT-2 data suggest that elotuzumab in combination with lenalidomide and dexamethasone achieved most favorable responses in patients with only one prior line of therapy after more than 3.5 years from primary diagnosis (Dimopoulos et al. 2017a). We thus compared PFS for patients from our cohort who received treatment within 3.5 years from their initial diagnosis to those who were exposed to treatment at later stages. Another subgroup analysis was performed for patients with more or less prior lines of therapy compared to the median number (4.5 lines). In both analyses, PFS did not differ significantly ($p \geq 0.71$; Fig. 2a, b).

We observed that patients with serum lactate dehydrogenase level above the normal range had progressed extraordinarily quickly. Therefore, we hypothesized that initial disease burden might potentially impact the efficacy of elotuzumab-based treatment. As surrogate parameter of disease load, we determined paraprotein levels at treatment initiation and divided the cohort in patients with levels below and above the median (median values—serum M spike: 22.3 g/l; serum IgA: 1464 mg/dl; urine free light chains: 1379 mg/24 h). The patient with IgD multiple myeloma was excluded from the analysis. Noteworthy, we observed a significantly longer PFS for patients with lower disease burden ($p = 0.02$; Fig. 2c). Surprisingly, patients with cytogenetically defined high and standard risk features (Chng et al. 2014) did not differ significantly in PFS ($p = 0.65$; Fig. 2d).

Association of NK-cell count with response to treatment

We next postulated that the number of circulating NK cells, the effector cells of ADCC, might play a relevant role for

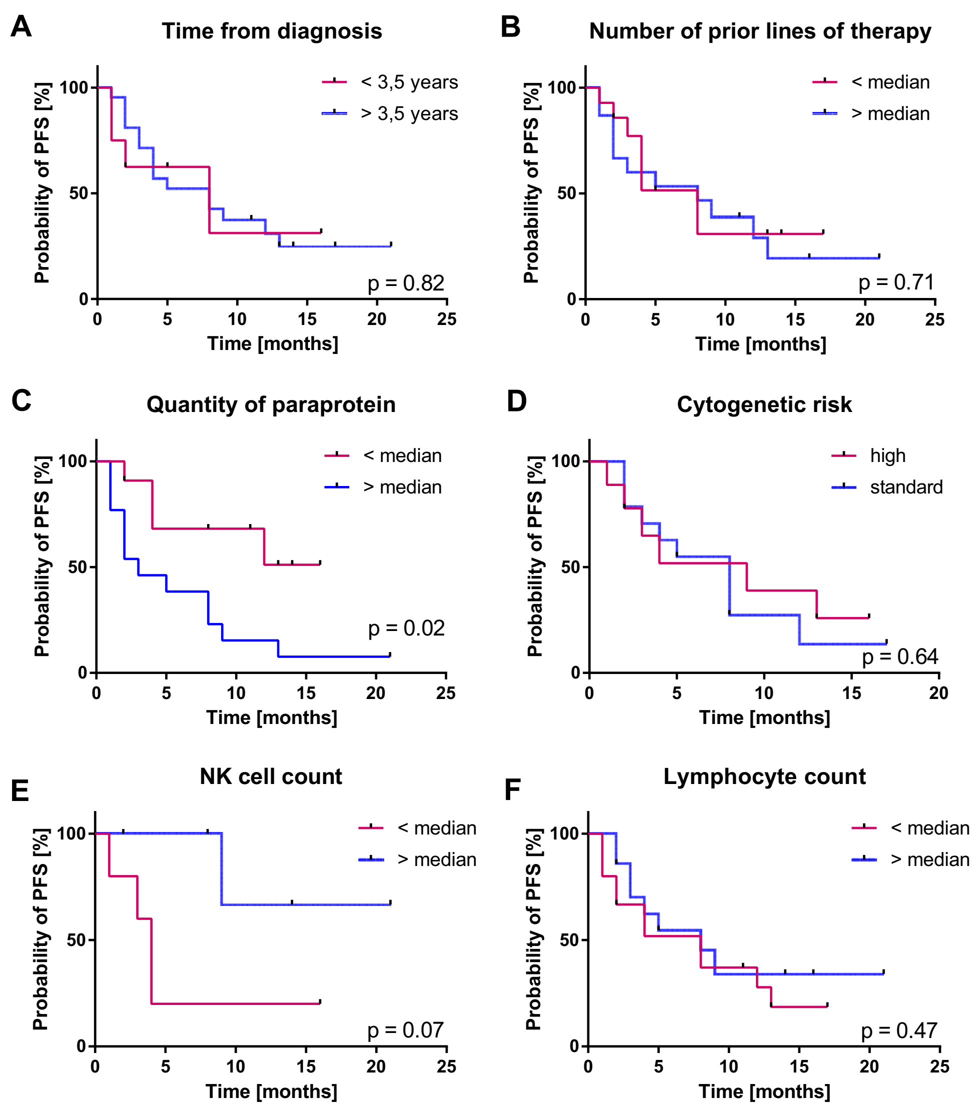

Fig. 2 Subgroup analyses of progression-free survival. Kaplan–Meier curves of progression-free survival (PFS) for patients: **a** treated before (red; median PFS: 8 months) or after (blue; median PFS: 8 months) 3.5 years after diagnosis; **b** pretreated with less (red; median PFS: 8 months) or more (blue; median PFS: 8 months) than the median of 4.5 prior lines of therapy; **c** starting treatment with lower (red; median PFS: not reached) or higher (blue; median PFS: 3 months) paraprotein levels than the median values (serum M spike: 22.3 g/l; serum IgA: 1464 mg/dl; urine free light chains: 1379 mg/24 h); **d** exhibiting cytogenetic high (red; median PFS: 9 months) or standard (blue; median PFS: 8 months) risk; **e** displaying lower (red; median PFS: 4 months) or higher (blue; median PFS: not reached) NK-cell counts than the median value of 334/µl; **f** displaying lower (red; median PFS: 8 months) or higher (blue; median PFS: 8 months) lymphocyte counts than the median value 1045/µl. *PFS* progression-free survival, *NK* natural killer

the efficacy of elotuzumab. In fact, a comparison of PFS in patients with NK-cell counts below or above the median (334/µl) prior to treatment unveiled a clear trend ($p = 0.07$) in favor of patients with high NK-cell numbers, despite the very limited sample size (ten patients evaluated; Fig. 2e). By contrast, total lymphocyte counts below or above the median (1045/µl) did not significantly influence duration of PFS ($p = 0.47$; Fig. 2f).

In aggregate, our data indicate that the efficacy of elotuzumab-based treatment depends on a favorable effector (NK) to target (myeloma) cell ratio.

Relapse with extramedullary disease

An indispensable prerequisite of successful targeted therapy is the expression of the respective antigen. As SLAMF7 confers homing of myeloma cells to the bone marrow niche (Tai et al. 2008), we analyzed the patients with disease progression for the development of extramedullary disease (EMD). 3 out of 19 patients (16%) relapsed with histologically proven EMD occurring in the maxillary soft tissue, in cervical lymph nodes and in the pleura, respectively. None of the patients had a history of EMD prior to elotuzumab treatment.

The patient with EMD in the pleura was a 70-year-old man diagnosed with IgG kappa multiple myeloma 16 years ago. After seven cycles of elotuzumab-based treatment, he developed symptomatic pleural effusion (Fig. 3a). He underwent diagnostic pleural puncture and simultaneous bone marrow aspiration 52 days after the last elotuzumab dose. In both compartments, CD38$^+$CD138$^+$ plasma cells were detectable by flow cytometry (Fig. 3b), however bone marrow infiltration was modest (20% plasma cells by immunohistochemistry). Staining for SLAMF7 expression unveiled a reduction of SLAMF7 expression on the plasma cells in the pleural effusion as compared to the bone marrow plasma

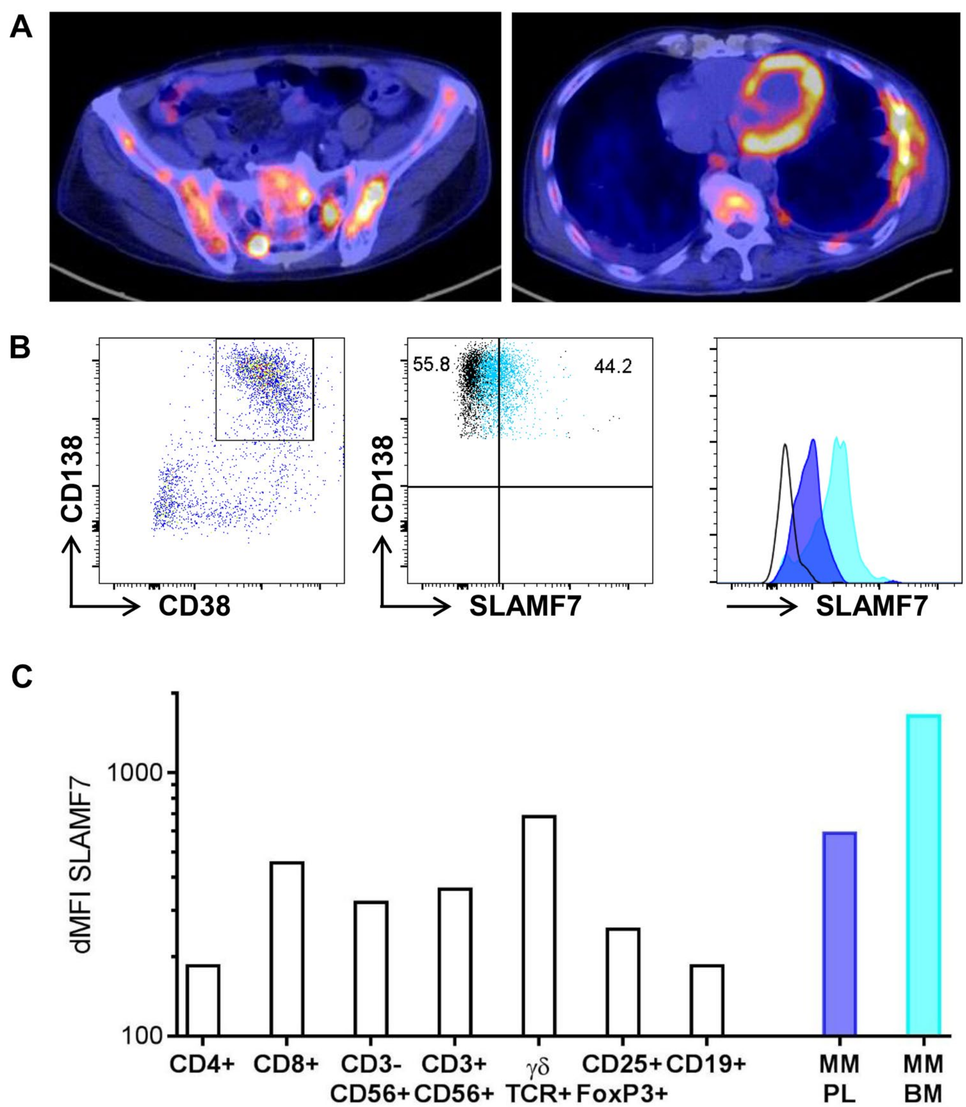

Fig. 3 Heterogeneity of SLAMF7 expression in a patient with extramedullary relapse after elotuzumab combination therapy. **a** 18F-FDG PET/CT imaging in a myeloma patient with symptomatic de novo left-sided pleural effusion demonstrates metabolically active intramedullary (left) and extramedullary lesions (right). **b** In bone marrow and pleural effusion, CD138$^+$CD38$^+$ plasma cells are identified by flow cytometry (left) and evaluated for SLAMF7 surface expression (middle). The histogram (right) depicts isotype control (black line), pleural (light blue) and bone marrow (dark blue) descended myeloma cells. **c** SLAMF7 expression on lymphocyte subsets, pleural (PL, light blue) and bone marrow (BM, dark blue) descended myeloma cells are depicted in differential mean fluorescence intensity (dMFI). *PL* pleural, *BM* bone marrow, *dMFI* differential mean fluorescence intensity

cells (Fig. 3b, c). Targeting SLAMF7 with elotuzumab might thus have promoted the development of a myeloma clone with less pronounced antigen expression and reduced homing to the bone marrow niche. As a BRAF V600E mutation was present in pleural and bone marrow descended myeloma cells, the patient was screened for inclusion into a phase I trial investigating the effect of pharmacological dual BRAF/MEK inhibition.

Taken together, time from diagnosis, number of prior lines of therapy and cytogenetic risk profile demonstrated little impact on treatment efficacy in our "real life" patient cohort. However, a favorable effector-to-target cell ratio—with high NK-cell numbers and low myeloma burden and strong expression of SLAMF7 on myeloma cells were biomarkers for an effective elotuzumab-based treatment.

Non-hematologic adverse events

Overall, elotuzumab-based treatment was well tolerated by most of the patients. Grade 3/4 non-hematologic toxicities occurred in 16 individuals (48%) and are depicted in Table 2. Infectious complications, including pneumonia (18%) and sepsis (6%) were the most frequent AEs. Only one grade 3 varicella zoster reactivation was noted. However, due to the high incidence of lymphopenic episodes, most patients (94%) received prophylactic acyclovir or famciclovir. One patient had detectable serum levels of hepatitis B virus DNA at start of elotuzumab treatment, received prophylactic entecavir and cleared the virus over time. Another patient contracted an acute hepatitis E infection (grade 1) during elotuzumab treatment, but had spontaneous virus clearance within 10 weeks of ongoing therapy. In summary, we did not observe a high rate of viral infection or reactivation.

Table 2 Non-hematologic adverse events

Grade 3/4	No. of patients (%)
Pneumonia	6 (18)
Sepsis	2 (6)
Upper respiratory tract infection	1 (3)
Device-related infection	1 (3)
Infectious enterocolitis	1 (3)
Febrile neutropenia	1 (3)
Mucosal infection	1 (3)
Herpes zoster	1 (3)
Maculopapular rash	1 (3)
Acute kidney injury	1 (3)
Abdominal pain	1 (3)
Diarrhea	1 (3)
Syncope	1 (3)
Hypertension	1 (3)

Hematologic adverse events

Blood counts of patients before and at nadir during elotuzumab treatment are visualized in Fig. 4a. For classification of hematologic AEs, only patients with initial blood counts corresponding to levels of grade 1 AEs or better (hemoglobin $\geq$ 10 g/dl, thrombocytes $\geq$ 75,000/µl, leukocytes $\geq$ 3000/µl, lymphocytes > 800/µl) dropping to levels of grade 3/4 AEs (hemoglobin < 8 g/dl, thrombocytes < 50,000/µl, leukocytes < 2000/µl, lymphocytes < 500/µl) were evaluated. While no grade 3/4 anemia and only two cases (7%) of grade 3/4 decrease in platelet counts were noted, grade 3/4 leukopenia occurred more frequently (25%). The most prominent grade 3/4 hematologic toxicity was decrease in lymphocyte count (63%).

We hypothesized that lymphopenia could be a SLAMF7-specific effect, as the elotuzumab target antigen SLAMF7 is known to be expressed on a variety of lymphocyte subsets (Gogishvili et al. 2017). By flow cytometry, we confirmed SLAMF7 surface expression on lymphocytes from healthy donors and myeloma patients prior to elotuzumab treatment (Fig. 4b, Supplementary Fig. 1). In both cohorts, SLAMF7 expression was most pronounced in NK cells ($CD3^-CD56^+$) and $CD8^+$ ($CD3^+CD8^+$), NK ($CD3^+CD56^+$) and $\gamma\delta TCR^+$ ($CD3^+\gamma\delta TCR^+$) T cells. Of note, no relevant SLAMF7 antigen was detectable on the surface of regulatory T (T_{reg}) cells ($CD3^+CD4^+CD25^+Foxp3^+$) in either cohort.

To further characterize treatment-emergent lymphopenia, we compared absolute cell counts of lymphocyte subsets before and after the first cycle of elotuzumab-based therapy (Fig. 4c). In contrast to observations from elotuzumab monotherapy (Zonder et al. 2012), we did not observe a significant reduction in NK-cell numbers; nor did we detect the previously described IMiD-mediated propagation of NK cells (Davies et al. 2001; Hsu et al. 2011). These data suggest that IMiD-induced increase in NK cells coincides with elotuzumab-associated NK-cell reduction. Given that mechanistically, NK cells are the central elotuzumab effector cells, an IMiD might thus be the optimal combination partner; the more so as high NK-cell numbers might be relevant for favorable responses. For the remaining lymphocyte subsets, we did not observe sole depletion of $SLAMF7^{high}$ lymphocytes ($CD8^+$ and $\gamma\delta TCR^+$ T cells), but also reduction of $SLAMF7^{low}$ subsets ($CD4^+$ T cells and B cells [$CD3^-CD19^+$]). Similar changes have previously been associated with IMiD/dexamethasone treatment (Paiva et al. 2016).

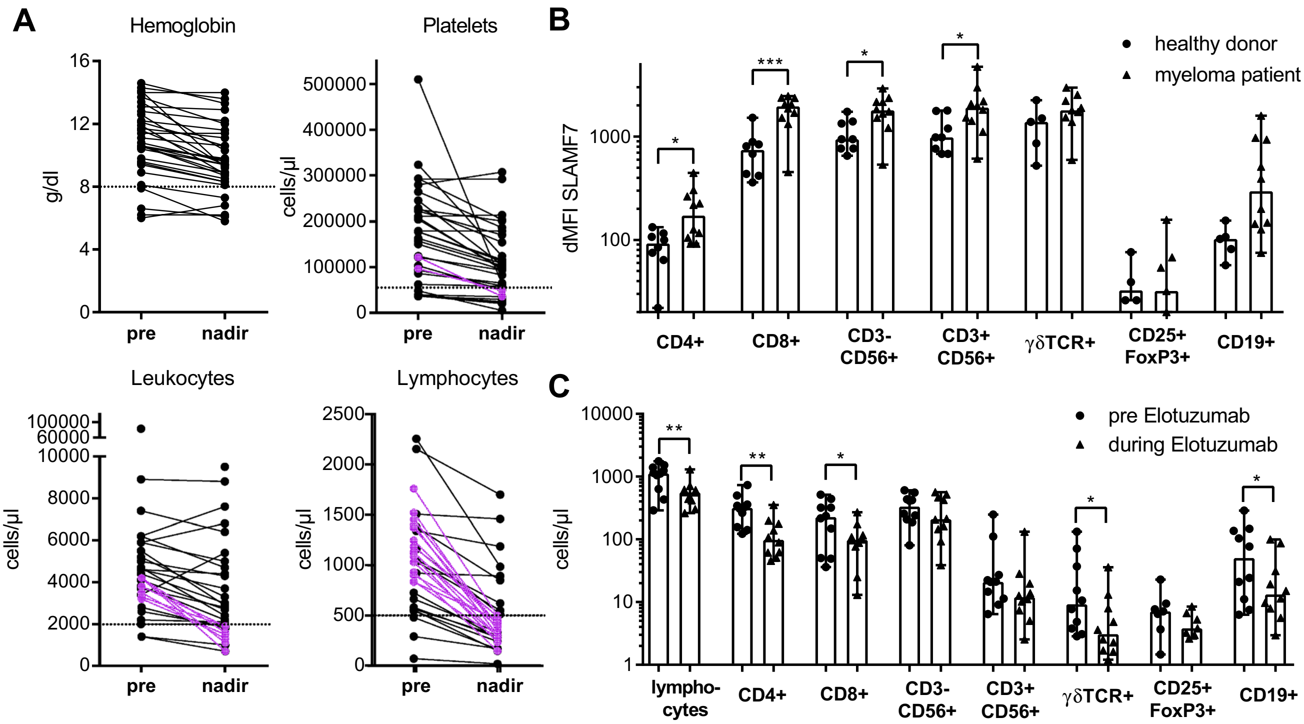

Fig. 4 Hematologic adverse events. **a** Hemoglobin, platelet, leukocyte and lymphocyte counts were evaluated before and throughout the elotuzumab combination treatment. Values prior to treatment initiation (pre) and at nadir are depicted. Patients with initial blood counts corresponding to levels of grade 1 adverse events or better (hemoglobin ≥ 10 g/dl, thrombocytes ≥ 75,000/µl, leukocytes ≥ 3000/µl, lymphocytes > 800/µl) dropping to levels of grade 3/4 adverse events (hemoglobin < 8 g/dl, thrombocytes < 50,000/µl, leukocytes < 2000/µl, lymphocytes < 500/µl) are highlighted in purple. **b** SLAMF7 surface expression on lymphocytes from healthy donors ($n = 8$) and myeloma patients prior to treatment initiation ($n = 10$) was evaluated by flow cytometry. Individual (dots and triangles) and median (bars) values of expression are depicted in differential mean fluorescence intensity (dMFI). Median dMFI (range)—CD4+$_{HD}$: 92 (22–133); CD4+$_{M}$: 172 (92–446); CD8+$_{HD}$: 743 (361–1519); CD8+$_{M}$: 1961 (454–2378); CD3-56+$_{HD}$: 942 (653–1739); CD3-56+$_{M}$: 1794 (536–2932); CD3+56+$_{HD}$: 983 (679–1791); CD3+56+$_{M}$: 1906 (611–4737); γδTCR+$_{HD}$: 1389 (524–2253); γδTCR+$_{M}$: 1796 (596–2978); CD25+FoxP3+$_{HD}$: 33 (26–76); CD25+FoxP3+$_{M}$: 32 (5–157); CD19+$_{HD}$: 102 (57–154); CD19+$_{M}$: 296 (75–1593). **c** Individual (dots and triangles) and median (bars) numbers of lymphocytes and lymphocyte subsets before (pre) and after completion of the first treatment cycle (during) are depicted. Median cells/µl (range)—lymphocytes$_{pre}$: 1130 (290–1760); lymphocytes$_{during}$: 562 (262–1300); CD4+$_{pre}$: 318 (140–730); CD4+$_{during}$: 98 (46–352); CD8+$_{pre}$: 226 (36–515); CD8+$_{during}$: 98 (13–271); CD3-56+$_{pre}$: 334 (80–607); CD3-56+$_{during}$: 209 (39–562); CD3+56+$_{pre}$: 21 (6–249); CD3+56+$_{during}$: 12 (3–133); γδTCR+$_{pre}$: 9 (3–133); γδTCR+$_{during}$: 3 (2–35); CD25+FoxP3+$_{pre}$: 7 (1–23); CD25+FoxP3+$_{during}$: 4 (3–8); CD19+$_{pre}$: 50 (7–136); CD19+$_{during}$: 13 (3–99). Statistical significance was evaluated using Student's *t* test and significance levels of $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***) were defined. *pre* prior to treatment, *dMFI* differential mean fluorescence intensity, *HD* healthy donor, *M* myeloma patient

Discussion

Based on data from the registration trial ELOQUENT-2 (Lonial et al. 2015), the monoclonal antibody elotuzumab was approved as first-in-class compound for the treatment of relapsed/refractory myeloma in combination with lenalidomide and dexamethasone. In the study population, the addition of elotuzumab to lenalidomide/dexamethasone increased the median PFS from 14.9 to 19.4 months. One major drawback of the trial is the low proportion of patients included with previous exposure to lenalidomide (5%). Very recently, elotuzumab was shown to have significant antimyeloma activity in conjunction with pomalidomide and dexamethasone even in heavily pretreated subjects (Dimopoulos et al. 2018). The latter trial reflects the approval of lenalidomide maintenance treatment in the US and Europe, on the basis of which lenalidomide is now being incorporated into routine first-line treatment in most patients. However, this "real-life" patient cohort is largely underrepresented in the ELOQUENT-2 study population. As the therapeutic options for myeloma patients at first, second or third relapse have increased dramatically with the availability of novel drugs including carfilzomib (Dimopoulos et al. 2016a, 2017b), pomalidomide (San Miguel et al. 2013), and daratumumab (Dimopoulos et al. 2016b; Palumbo et al. 2016), treating physicians are progressively facing the torment of choice. Therefore, growing efforts are undertaken to define patient subgroups who benefit most from a certain treatment regimen. However, due to selective inclusion criteria, many study populations may insufficiently reflect the regular patient collective.

In our patient cohort, the PFS was considerably shorter as compared to the ELOQUENT-2 population. This might be due to prior exposure to IMiDs in all but one patient which had been extensive in some cases. More than one-third had acquired refractoriness to at least one IMiD as part of a multi-drug regimen, whereas only one patient had received the IMiD as maintenance treatment before initiation of elotuzumab-based therapy. Furthermore, two-thirds of patients either started on reduced doses of IMiDs or required dose reductions throughout the course of the treatment due to organ toxicities.

In the ELOQUENT-2 trial, two subgroups with favorable response to elotuzumab-based treatment were identified: patients diagnosed more than 3.5 years before initiation of elotuzumab treatment and patients with only one prior line of therapy. In our "real-life" cohort, patients received elotuzumab-based therapy at a median of 68 months after diagnosis and after a median of 4.5 prior lines of therapy. Patients thus received elotuzumab considerably later than the study population from ELOQUENT-2. In this small cohort, the patients treated at more than 3.5 years from their primary diagnosis or following a below average number (≤ 4) of prior lines of treatment could not be identified as favorable responders.

However, we postulated that for efficient disease control by targeted immunotherapy, biological disease characteristics might be relevant. We observed that both the tumor burden as assessed by paraprotein production and the number of NK cells prior to treatment initiation can influence the efficacy of the antibody treatment. This observation was made under the assumption of a positive linear correlation between monoclonal serum or urine protein and burden of myeloma cells (Salmon and Smith 1970). Further, we did not evaluate the functional status of NK cells. Naturally, paraprotein level and NK-cell count may be surrogate parameters for advanced disease stage with poor prognosis (Barlogie et al. 1983). However, albeit previously associated with adverse outcomes (Chng et al. 2014; Jimenez-Zepeda et al. 2015), neither cytogenetic risk stratification by FISH nor absolute lymphocyte numbers seemed to influence the duration of PFS in this small cohort. Whether a debulking strategy to reduce tumor burden could restore a favorable response even in patients with high disease load requires further investigations; especially as many cytoreductive therapies have lymphodepleting side effects and can thus reduce NK-cell counts.

The case of a patient with pleural myeloma progression during elotuzumab treatment demonstrated that the presence of the antigen is the indispensable prerequisite for efficacy of targeted therapy. Therefore, assessment of the expression of the target antigen might be justified prior to treatment initiation; especially if the functional role of the antigen is abdicable, as is the bone marrow homing factor SLAMF7 in EMD.

In accordance with previous studies, ELOQUENT-2 demonstrated a favorable safety profile for the triplet combination of elotuzumab and lenalidomide/dexamethasone. However, the most frequent grade 3/4 AE was a decrease in lymphocyte count that remained below the levels of the lenalidomide/dexamethasone control cohort throughout the treatment (Lonial et al. 2015). For elotuzumab monotherapy reversible reduction in NK-cell numbers was documented (Zonder et al. 2012), but the effects of the elotuzumab/IMiD/dexamethasone combination on distinct lymphocytes subsets have not been investigated in detail.

We and others have previously demonstrated that T- and B-cell numbers are reduced during lenalidomide treatment (Clave et al. 2014; Danhof et al. 2017; McCarthy et al. 2012). The changes in lymphocyte counts after the first treatment cycle in our cohort did not correspond to the surface expression of SLAMF7 on the lymphocyte subsets. However, these changes largely correlated with IMiD/dexamethasone-associated alterations. Therefore, in the setup of this single-arm study, we could not carve out SLAMF7-specific fratricide of other SLAMF7^{high} lymphocyte subsets by elotuzumab.

In this respect, we found it interesting that, for regulatory T cells, we did not detect relevant SLAMF7 surface expression. Furthermore, elotuzumab treatment did not significantly alter numbers of regulatory T cells. This contrasts with the mode of action of daratumumab that includes depletion of CD38^{+} regulatory T cells, induction of T-cell expansion and promotion of antiviral and alloreactive functional responses (Krejcik et al. 2016).

IMiDs have been known to activate and expand NK cells in vivo for a long time (Davies et al. 2001). In our cohort, no change in NK-cell numbers was apparent. Taken together, our data are in line with previous analyses: the NK-cell propagating effect of IMiDs seems to overcome the elotuzumab-associated NK-cell reduction, resulting in stable NK-cell numbers. Besides its direct antimyeloma activity, the IMiD-elotuzumab combination thus acts synergistically by expanding (IMiD), activating (IMiD and elotuzumab) and redirecting (elotuzumab) NK cells for the elimination of myeloma cells.

In summary, the selection of defined patient subgroups with high probability of favorable outcomes may improve efficacy of elotuzumab-based treatment. It might thus be advisable to use elotuzumab-based therapy preferentially in patients with low disease burden and high NK-cell numbers; as well as in patients with high expression of SLAMF7 on intra- and extramedullary myeloma lesions. However, the predictive value of disease burden, NK-cell count and SLAMF7 expression on myeloma cells requires further evaluation in larger prospective clinical trials.

Author contributions SD, SK and MS designed the research study; SD, SS, DH and MS performed the research; SS, MB, HE and MS contributed essential research material; SD, MK, MB and JH analyzed the data; HE supervised the project; SD drafted the manuscript; SS, DH, MK, MB, JH, HE, SK and MS carefully revised the manuscript; all authors approved the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest SK: honoraria from Celgene GmbH and Bristol-Myers Squibb GmbH. SD, MS: Advisory Board for Bristol-Myers Squibb GmbH.

Ethical Approval for Research involving Human Biospecimens All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants for whom biospecimens were included in the study.

Ethical approval for research involving animals This article does not contain any studies with animals performed by any of the authors.

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