Clinical Case Reports

Open Access

CASE REPORT

Fulminant onset of acute leukemia from normal hematopoiesis within 3 months of follow up for multiple myeloma treated with total therapy protocols

Zeba N. Singh[1,a], Yogesh Jethava[2], Ginell R. Post[1], Daisy Alapat[1], Jeffrey Sawyer[1], Sarah Waheed[2], Bijay Nair[2,b], Saad Z. Usmani[2,c], Clyde Bailey[2], Nathan Petty[2], Frits Van Rhee[2] & Bart Barlogie[2]

[1]Department of Pathology, University of Arkansas for Medical Sciences, 4301 W. Markham Street Slot 502, Little Rock, Arkansas, 72205
[2]Myeloma Institute for Research and Therapy, University of Arkansas for Medical Sciences, 4301 West Markham Slot 816, Little Rock, Arkansas, 72205

Correspondence
Zeba N. Singh, Department of Pathology, University of Maryland Medical Center, Baltimore, MD 21201. Tel: 410-328-2146; Fax: 410-328-5508; E-mail: zsingh@umm.edu

Present address
[a]Department of Pathology, University of Maryland Medical Center, Baltimore, Maryland, 21201
[b]9600 Baptist Health Drive Suite 200, Little Rock, Arkansas, 72205
[c]Levine Cancer Institute/Carolinas Healthcare System, 200 S College St #500, Charlotte, North Carolina, 28202

Funding Information
This work was supported in part by a grant from the National Cancer Institute, National Institutes of Health (grant number CA 55813).

Received: 29 May 2014; Revised: 2 October 2014; Accepted: 25 October 2014

Clinical Case Reports **2015; 3(3): 183–192**

doi: 10.1002/ccr3.180

Key Clinical Message

Assiduous surveillance for genetic aberrations is necessary in patients on cytotoxic therapies to detect therapy-related myeloid neoplasms (t-MN). Current modalities include metaphase cytogenetics and FISH. Since t-MN may develop abruptly in cytogenetically normal patients, a discussion exploring additional methods such as SNP-array and targeted-deep-sequencing to detect subchromosomal abnormalities is needed.

Keywords

Acute leukemia, cytogenetic abnormalities, myeloma, therapy-related myeloid neoplasm.

Introduction

The development of secondary malignancies following high-dose chemo-radiotherapy (HDT) in multiple myeloma (MM) is well established [1–3]; recent reports have described an association with IMiD therapy as well [4, 5]. Based on a large population-based study [6] therapy-related myeloid neoplasms (t-MN) are the most common second malignancy in MM patients on therapy, with a standardized incidence ratio of 11.51 (CI 8.19–15.74). We and others, have previously shown that clonal, myelodysplasia-associated cytogenetic abnormalities (MDS-CA) may be transiently observed during the course of chemotherapy, and do not always correlate with presence of morphological dysplasia, or require specific therapeutic intervention. [5, 7, 8] However, most

patients who have persistent or progressive cytopenia, and eventually develop t-MN, harbor persistent cytogenetic abnormalities [1–3]. In a recent paper, [5] we described a number of pretreatment host-, and treatment-related variables that could be linked to development of MDS-CA, such as advanced age, longer duration of pretransplantation chemotherapy, low CD34 yield, and more leukapheresis sessions to obtain the desired quantity of hematopoietic progenitor cells. In this study, we draw attention to fulminant onset of acute leukemia (FOAL) in patients who did not have any previously identified significant morphological dysplasia or MDS-CA, and had a morphologically and karyotypically normal staging bone marrow (BM) within the previous 3 months. We searched for FOAL-defining features in the context of the more common therapy-related acute leukemia (t-AL) arising with prodromal clinical features or MDS-CA.

Patients and Methods

Patient cohort

Our MM database was scrutinized for all patients who had received hematopoietic progenitor cell-supported HDT at our institution since 1989. Only patients who had received at least one HDT regimen and an autologous hematopoietic stem-cell transplantation (auto-HSCT), and had informative cytogenetic data, defined as at having least one evaluable karyotype before, and one at a minimum of 28 days after HDT, are included in the study. All participating patients had signed an initial informed consent in keeping with institutional, federal, and international guidelines. A subset of the patients in this cohort (patients on TT2, TT3a, and TT3b protocols) was included in a recent publication on risk factors for myelodysplasia [5]. This study includes all qualifying MM patients who received any of the following: Total therapy protocols (TT-P) for newly diagnosed MM [9–12] non-TT protocol, or off-protocol therapies. BM examinations and cytogenetic studies were performed for all patients at diagnosis, at 3-month intervals during the first year of maintenance, every 6 months for 3 years, and annually thereafter.

Definitions

CA commonly observed in myelodysplasia or acute leukemia [13] are referred to as MDS-CA in this study. We define MDS-CA as "persistent" when the same *clonal* chromosomal abnormality was observed in three successive metaphase cytogenetic analyses, and as "transient" when the *clonal* abnormalities were observed in less than three successive analyses, or when the abnormalities observed in the successive evaluations were dissimilar [8]. Since chemotherapy *per say* can result in morphological dyspoiesis, to avoid confounding bias, we identify a patient as having developed t-MN only when associated with persistent MDS-CA. Patients who required management of cytopenias with disease-modifying therapies such as hypomethylating agents including decitabine and 5-azacytidine, or cytotoxic chemotherapy, which is typically a combination of doxorubicin, Ara-C, and melphalen (DAM), or HSCT with BEAM conditioning, are designated as clinical MDS (C-MDS). Patients who received transfusion support, erythropoiesis-stimulating agents, or myeloid growth factors are excluded from the C-MDS because these management modalities are frequently used to facilitate recovery from cytopenia following HDT or HSCT. The FOAL group has already been defined. Acute leukemia developing following HDT is referred to as t-AL (AML/ALL). Patients with t-MN who had less than 20% blasts in the BM or peripheral blood are termed as t-MDS in this study. Complete remission is defined by the international myeloma working group (IMWG) criteria. [14].

Morphology, flow cytometry, and cytogenetics

A diagnosis of FOAL pertained to eight patients who, within 3 months of their last thorough evaluation at our institution, qualified as having no clinical, morphological, or genetic evidence indicative of MDS. All BM specimens within the one-year period prior to FOAL were reevaluated. Morphological evaluation of myelodysplasia was based on previously described parameters [15, 16] and performed by three experienced hematopathologists (Z. S., G. P., D. A.). Flow cytometry was performed on BM samples for quantitation and immunophenotypic characterization of the blasts. Metaphase cytogenetic analysis by GTG banding was performed on unstimulated BM specimens using standard techniques. At least 20 metaphases were examined from each specimen. The cytogenetic nomenclature followed standard ISCN criteria [17]. Interphase FISH was performed using probes to detect abnormalities commonly observed in MDS and AML such as −5/del(5q), −7/del (7q), del(20)(q11.2), +8, and −13. Additional probes were used in some patients at the onset of FOAL to detect translocations involving 11q23/*MLL* gene, t(8;21), t(15;17), inv (16) or t(16;16), t(9;22), and t(8;14).

Results

Of the 3941 patients undergoing mainly melphalan-based HDT, 391 patients developed MDS-CA (Fig. 1). Since the emphasis of this work is on t-MN, the 56 patients who

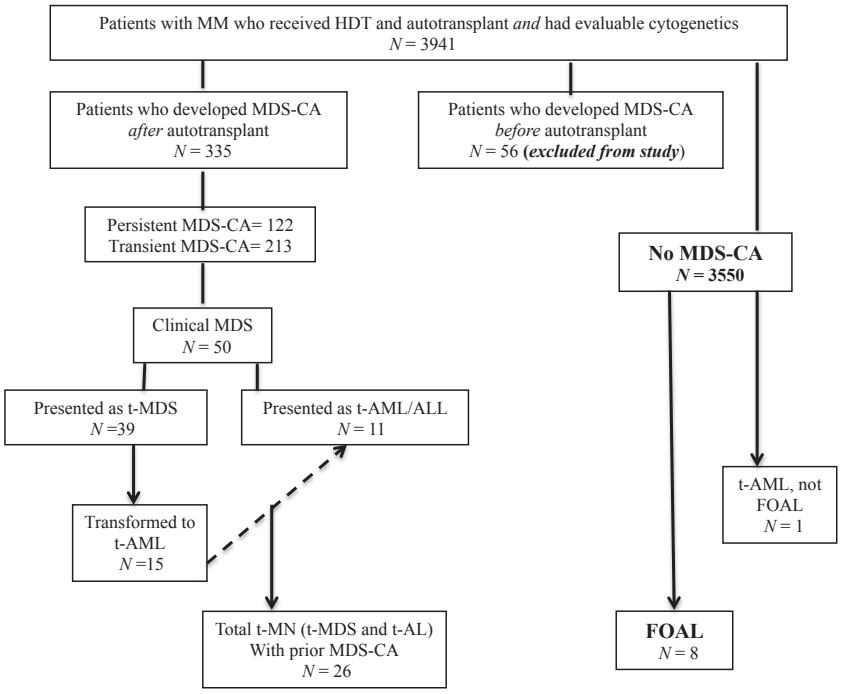

Figure 1. Study cohort showing the distribution of patients according to presence of MDS-CA, the development of t-MN (t-MDS or t-AL [AML or ALL]), and of FOAL. MDS-CA, myelodysplasia-associated cytogenetic abnormalities; t-MN, therapy-related myeloid neoplasms; t-AL, therapy-related acute leukemia; FOAL, fulminant onset of acute leukemia.

exhibited MDS-CA *prior* to first transplant were excluded from further analysis. Of the 335 remaining patients, 213 showed transient MDS-CA and 122 had persistent MDS-CA. Fifty patients with t-MN requiring disease-modifying therapies qualified as C-MDS. Of these 50 patients, 11 presented with t-AL (t-AML (10); t-ALL(1)) and 39 with t-MDS. Fifteen of the t-MDS patients subsequently progressed to t-AL. Of the 3550 patients who *did not* have MDS-CA, nine developed t-AL: eight of them qualified for a diagnosis of FOAL as previously defined; one patient who had persistent, clinically significant cytopenia prior to onset of leukemia, was not included in the FOAL group.

Characteristics and treatment of FOAL patients

The baseline MM parameters of FOAL patients are outlined in Table 1. Their age ranged from 41 years to 66 years, six were males and two females. The immunoglobulin distribution of the MM was representative of our referral population. The time from MM diagnosis ranged from 4 to 12 years. Myeloma-associated CA (MM-CA) were present in three of the eight patients at diagnosis of MM. Gene expression profiling (GEP)-defined molecular subgroups [18] were representative of newly diagnosed patients. Both GEP-70 and GEP-80 risk scores [19] were low in seven patients, while one had high-risk features in both models. Treatment regimes included TT2 without thalidomide in 1, TT2 with thalidomide [9] in 2, TT3a (maintenance with bortezomib, thalidomide, and dexamethasone) [10] in 3, TT3b (maintenance with bortezomib, lenalidomide, and dexamethasone) [11] in 1, and TT4 (similar to TT3b for GEP-defined low-risk MM) [12] in one patient. Table 2 summarizes the clinical, cytogenetic, morphological, and immunophenotypic features of patients with FOAL at onset of t-AL. The complete blood count (CBC) values over the last 12–18 months (at 3- to 6-month intervals) were as follows: hemoglobin 12.8 g/dL (range 7.8–13.6 g/dL; mean 12.0 g/dL), WBC 3.52 K/μL (range 1.17–9.7 K/μL; mean 4.38 K/μL), and platelet count 147 K/μL (range 60–280 K/μL; mean 157 K/μL). Latency periods to FOAL diagnosis from treatment initiation, and from first HDT, ranged from 11 to 134 months and 6–118 months, respectively (Table 2). The MM status at onset of FOAL was stringent complete response (sCR) in seven patients and complete response (CR) in one patient. Seven of the eight FOAL patients were classified as t-AML and 1 as t-precursor B-ALL. The morphology and immunophenotype from representative t-AML and t-ALL cases are illustrated in Figures 2A–C and 3A–C, respectively.

Table 1. Baseline myeloma parameters in FOAL patients.

Patient no.	1	2	3	4	5	6	7	8
Parameters								
Age/Sex	41/M	44/M	59/M	54/M	61/M	66/F	48/F	66/M
Race	AA	W	W	W	AA	W		
MM Ig subtype	IgG K	IgG L	K light chain	Nonsecretory K light chain	K light chain	IgA K	IgG K	IgG K
Date of diagnosis	09/2005	08/2009	01/2001	05/2005	03/2001	09/2005	12/2001	09/2006
Risk-score at Diagnosis	70-gene: Low 80-gene: Low	70-gene: Low 80-gene: Low	70-gene: Low 80-gene: Low	70-gene: High 80-gene: High	70-gene: Low 80-gene: Low	70-gene: Low 80-gene: Low	70-gene: NA 80-gene: NA	70-gene: Low 80-gene: Low
Molecular subtype	CD2	LB	Hyperdiploidy	Proliferation subset with LB	LB	MS	HY	NA
Karyotype at diagnosis of MM	46,XY[20]	46,XY[20]	46,XY[20]	38~40,X, −Y,+1, add(1)(p11), der(1;16)(q10;p10), −4,−6, t(8;14)(q24.1;q32),−8,−12, −13,−16 [cp4]/ 70~75,idemx2, inc[cp3]/ 46,XY[cp13]	46,XY[20]	42~44,X,−X, add(6)(q?22), der(12)t(1;12)(q11~12;q24.3), -13 [cp9]/46, XX[cp11]	53,XX,+3,+5,+7, +9,+9,+11,+15, +19,−22[1]/ 46,XX[12]	46,XY[20]
Treatment protocol	2003-33 (TT3) randomized to VTD arm	2008-01 (TT4) randomized to lite arm	98-026 (TT2) randomized to T-arm	2003-33 (TT3) randomized to VTD arm	98-026 (TT2) randomized to T-arm	2003-33 (TT3) randomized to VTD arm	98-026 (TT2) randomized to non-T-arm	2006-66 (TT3)
Lenalidomide: Yes/No, duration	Yes, 7 months	No	No	Yes, 30 months	No	Yes, 4 months	No	Yes, 6 months
No. of HSCT	2	2	2	2	2	2	3	3
Preparatory regimen	Mel 200 Mel 200	Mel 200 Mel 200	Mel 200 Mel 200	Mel 200 Mel 200	Mel 200 Mel 200	Mel 200 Mel 200	Mel 200 BEAM Mel 200, Gemcitabine	Mel 200 Mel 200
Stem cells collected $>3 \times 10^6$	No	No	Yes	No	No	No	No	No
>2 apheresis	No	No	Yes	No	No	No	No	No

Table 2. Clinical, cytogenetic, morphological, and immunophenotypic characteristics of the FOAL group 3 months prior to, and at the onset of acute leukemia.

Patient no.	1	2	3	4	5	6	7	8
Parameters								
Latency period from start of chemotherapy to FOAL	66 months	11 months	134 months	75 months	127 months	77 months	68 months	65 months
Latency period from last transplant to FOAL	52 months	6 months	118 months	71 months	116 months	68 months	25 months	60 months
MM status at FOAL	sCR	sCR	CR	sCR	sCR	sCR	sCR	sCR
Karyotype within 3 months prior to FOAL	46,XY[20]	46,XY[20]	46,XY[20]	46,XY[20]	46,XY[20]	46,XX [20]	46,XX [20]	46,XX//XY[19]/ 46,XY,del(20)(q11.2)[1]
FISH within 3 months prior to FOAL	ND	Normal	Normal	ND	ND	ND	Normal	Normal
BM within 3 months prior to FOAL	Normocellular, No dysplasia, no residual MM	Normocellular, No dysplasia, no residual MM	Normocellular, No dysplasia, no residual MM	Normocellular, No dysplasia, no residual MM	Normocellular, No dysplasia, no residual MM	Normocellular, No dysplasia, hematogones 10%, no residual MM	Normocellular, No dysplasia, no residual MM	Mildly hypocellular with rare hypolobate megakaryocytes
Karyotype at time of FOAL	46,XY[20]	46,XY[20]	43~44,XY, +3,add(3)(p13), **del(3)(q12),**−**5,**−**7**, ?t(8;10) (q22;q26), −16,−**17**,−18,inc[cp15] / 46,XY[25]	39~44,XY**,**−**5,**−**7**, **der(12)t(12;?17)(p13;q21)**, −16, add(17)(p11.2), −18,−19, +2mar[cp40]	42~43,XY, der(3;12)(q10;q10), del(4)(q31.3), ucode>−**5**,−6, der(14)t(11;14)(q12~13.1;q32) **del(11)(q23)**, **inv(16)(p?13.1q?22),** −**17**,+mar[cp11]/ 42~43, idem, del(1)(q21), ?inv(7)(p21q11.1)[cp9]/46,XY[1]	46,XX,del(9)(p12), **del(20)(q11.2q13.1)** [2]/46,XX[cp38]	43~52,XX,der(1;16)(q10;p10), add(10)(q26), del(11)(p11.1), add(?13) (q34), del(13)(q12q22), del(16)(q22), add(17)(p13), **del(20)(q11.2)**, +1~3mar,2~3dmin, inc[cp14]/46,XX[6]	46,XY,**t(3;8)(q27;q24.1),** −**7**,+mar[4]/45, idem,-mar[6]/ 46,XY[10]
FISH	ND	Normal	del(5q31),−7, del(7q)	−5, del(5q31), −7, +21q22, +17q21, del (16q22), +9q34, −13	del(5q31)	del(20q)	Normal	−7,del(7q)

(*Continued*)

Table 2. Continued.

Patient no.	1	2	3	4	5	6	7	8
Other molecular tests	ND	ND	ND	Negative for JAK2 V617F	Negative for the FLT3 Internal Tandem Duplication, FLT3 D835 variant; and NPM1 mutation	ND	ND	ND
FOAL subtype	AML	AML	AML	AML	AML	Precursor B-ALL	AML	AML
BM at FOAL	BM not reviewed at our institution. Details NA	Hypercellular, blasts 75%; Decreased megakaryopoiesis and erythropoiesis	Normocellular (35%), blasts 20%, No significant dyspoiesis	Hypercellular, blasts 32%, marked trilineage dysplasia	Hypercellular, blasts 85–90%, markedly decreased Erythropoiesis and megakaryopoiesis, marked dysgranulopoiesis on peripheral blood	Normocellular, blasts 35%; trilineage dysplasia	Hypercellular, blasts 40%; erythroid and Megakaryocytic dysplasia, mild granulocytic dysplasia.	Hypercellular, blasts 65%; dysmegakaryopoiesis
Immunophenotype	Details not available	Positive: CD33, CD15, CD13 (variable), HLA-DR, CD14, CD11b, CD56 (subset), and CD4(dim)	Positive: CD34, CD117, CD13, CD33, CD7, CD5, and MPO, and CD4 (partially, weak)	Positive: CD45 (dim), CD34, CD117, CD15, CD33, CD13, CD56, and HLA DR	Positive: CD45 (dim), CD34, CD117, CD33, CD13, CD56 (dim), and HLA-DR	Positive: CD34, TdT, CD19, CD10, CD20, CD99, CD44	Positive: HLA-DR, CD117, CD33, CD13	Positive: CD34, CD117, HLA-DR, CD33, CD13, CD56, CD5, and CD7
		Negative: CD34, CD117, cMPO	Negative: HLA-DR, TdT, CD14, CD56, CD15, glycophorin-A, and all lymphoid antigens .	Negative: CD3, CD19, MPO, TdT, and other tested myeloid, and lymphoid antigens	Negative: CD3, CD19, CD15, CD11b, all other myeloid antigens, and lymphoid antigens.	Negative: CD117, CD33, CD13, CD15, MPO, all T-lymphoid antigenss	Negative: CD34, and all myeloid and lymphoid antigens	Negative: cMPO,, all other myeloid and lymphoid antigens (except D5 and CD7)
Acute leukemia therapy	Clofarabine, Idarubicin, Cytarabine	Idarubicin, ara-C, etoposide, and fludarabine	Decitabine, followed by Decitabine + Azacytidine + Melphalen	Decitabine, Allogenic HSCT	7 + 3 induction with idarubicin, ara-C; Decitabine + Azacytidine + Melphalen	Hyper-CVAD with Rituxumab and inotuzumab	ara-C, VP-16, Mylotarg	Idarubicin, ara-C, and dexamethasone
Response	Refractory AML	In CR for t-AML and MM for 3 months	In CR for t-AML and MM for 2 months	CR t-AML and MM	In CR for 5 months, then relapsed	In CR for t-ALL and MM for 3 months	In CR for t-ALL and MM	In CR for t-ALL and MM for 2 months
Survival in months after FOAL	11	17	6	11	5	9	38	2
Outcome	Death	Death	Alive	Death	Alive	Alive	Death	Alive

The bold text highlights the cytogenetic abnormalities commonly associated with myelodysplasia.

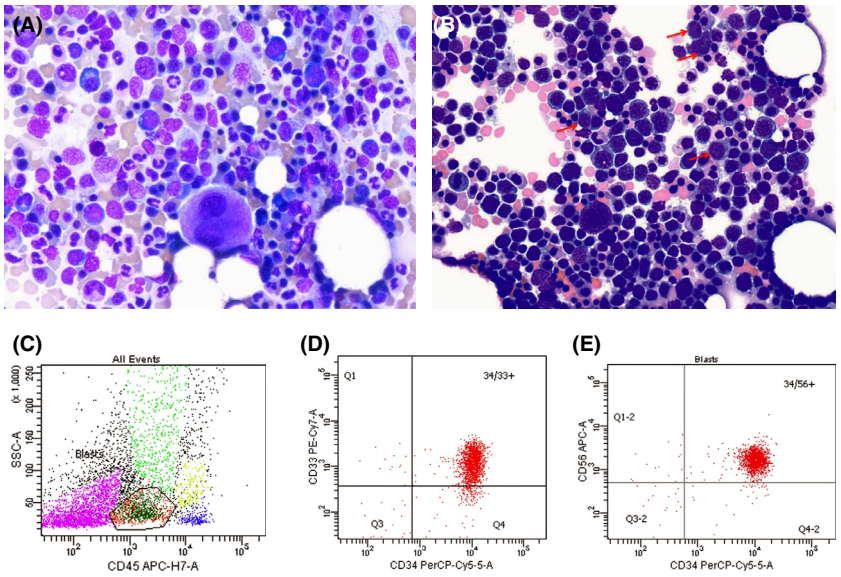

Figure 2. (A) BM aspirate smear from patient no.4, 3 months prior to FOAL showing normal trilineage hematopoiesis without dyspoiesis (Wright-Giemsa, 200X). (B) BM aspirate smear from same patient at onset of FOAL (t-AML). Red arrows point to leukemic blasts (Wright-Giemsa, 200X). (C) Flow cytometry of BM sample from same patient at FOAL with the gated dim CD45+ blasts (C) blasts coexpress CD34, CD13, and CD56 (D and E). FOAL, fulminant onset of acute leukemia; BM, bone marrow; t-AL, therapy-related acute leukemia.

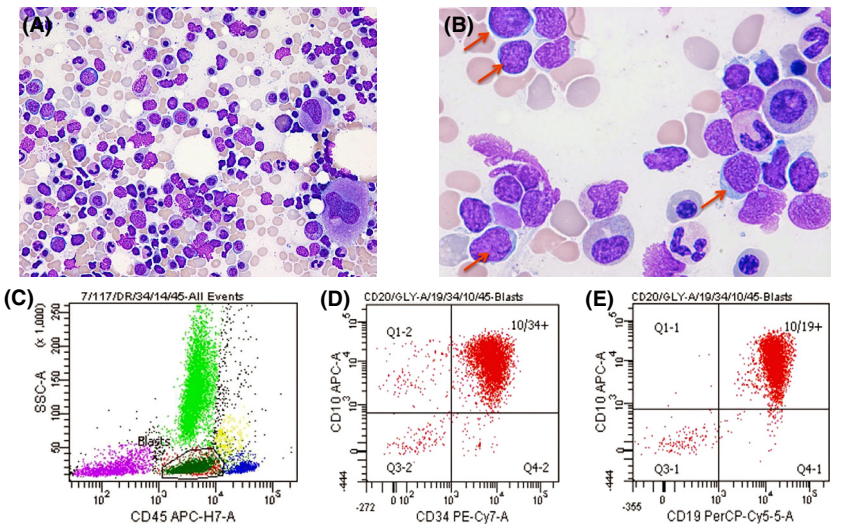

Figure 3. (A) BM aspirate smear from patient no.6, within 3 months prior to FOAL showing normal trilineage hematopoiesis without dyspoiesis (Wright-Giemsa, 200X). (B) BM aspirate smear from same patient at onset of FOAL (t-precursor B-ALL). Red arrows point to leukemic blasts (Wright-Giemsa, 400X). (C) Flow cytometry of BM sample from same patient at FOAL with the gated dim CD45+ blasts (C) blasts coexpress CD34, CD19, and CD10 (D and E). FOAL, fulminant onset of acute leukemia; BM, bone marrow; t-AL, therapy-related acute leukemia.

None of the patients had MM-related karyotypic abnormalities in the year preceding the onset of FOAL, or any MDS-CA by conventional metaphase cytogenetic study or FISH, at any time prior to the development of FOAL. FISH data available in four patients during the 3-month-period prior to FOAL, did not identify MDS-CA with the employed probes. In one patient, del (20)(q21) was identified transiently in 1 of 20 metaphases, which does not qualify as clonal. At onset of FOAL, the karyotype in this patient did not show the del(20)(q21) abnormality (Table 2). At the *onset* of FOAL, clonal MDS-CA were present in six of eight patients, in five of whom the karyotype was highly complex. Complete or partial deletions of chromosomes 5 or 7 were

observed in three and three patients, respectively, and abnormalities of both 5 and 7 were present in two patients. The MDS-CA identified by FISH in four of the seven cases examined matched those observed by metaphase karyotyping.

Treatment of FOAL was individualized and was based on the patients' comorbidities and performance status (Table 2). Seven of the eight patients achieved CR, which was short-lived, ranging from 2 to 17 months.

Discussion

The development of secondary malignancies following HDT in multiple myeloma (MM) is well established [1–3]; recent reports have described an association with IMiD therapy as well [4, 5]. In our population of 3941 patients who received HDT, 8.5% developed MDS-CA, which were transient in the majority (two-thirds) of the patients. A subset of patients with MDS-CA required therapy (C-MDS); of these patients, 39 fulfilled diagnostic criteria for t-MDS and 11 were classified as t-AL (group C). Of those patients with t-MDS, 15 transformed to t-AML (group B-indicate). In contrast, FOAL was observed in eight patients without antecedent MDS-CA, significant morphologic dysplasia or clinically significant cytopenias (group A). The classic form of t-MN that develops following heavy prior treatment with alkylating agents, is usually associated with a prior myelodysplastic phase marked by cytopenia, and significant morphological dysplasia in the BM, often involving all three cell lines [1–3]. In this respect the FOAL patients, all of whom were heavily pretreated with melphalen, an alkylating agent, had an unusual presentation.

We attempted to identify predictive features for FOAL vis-à-vis patients with MDS-CA who presented as t-AL (11 patients; group C) or developed t-AL from antecedent t-MDS (15 patients; group B). There were no differences among the three groups with respect to age, sex number of transplants received or latency phase to t-AL. In our previous study on a large cohort of patients [5], and also from a recent paper by Pemmaraju et al. from M.D. Anderson cancer center [20], data highlights advanced age, longer duration of pretransplantation chemotherapy, low CD34 yield, and more leukapheresis sessions to obtain the desired quantity of hematopoietic progenitor cells as predictive of t-MN. Interestingly, 3 (6.4%) patients described by Pemmarju and others developed therapy-related chronic myelomonocytic leukemia, not a commonly described t-MN. Forty-three of the 46 patients (93%) who developed t-MN in their study had cytogenetic abnormalities, with high-risk abnormalities in 78% patients, but no patients are described to have an abrupt onset of acute leukemia without prior t-MDS or CA as observed by us. In fact, in our review of literature no specific mention of such a catastrophic development of t-AL has been previously made. This is especially remarkable because although the FOAL patients did not have any prior MDS-CA, interestingly, the proportion of patients with high-risk MDS-CA including −7, del(17)(p13)/*TP53* and complex karyotypes (>3 abnormalities), were higher in this group (FOAL) of patients at the onset of t-AL compared with the two other groups in our study. There was no difference in survival from onset of t-AL among the patients groups (medians of 14, 9, and 10 months, respectively), nor did the latency duration affect survival in any of the groups of t-AL (data not shown). Notably, all FOAL patients had received TT protocols compared with five of 11 of group B (non-TT-P- 1; off-P, 5), and seven of 15 of group C patients (non-TT-P, 4; off-P, 4). Of all the parameters evaluated, we noted that patients with prior exposure to lenalidomide had a higher incidence of developing FOAL; four of eight patients in the FOAL group had prior lenalidomide exposure, which is higher in incidence than the two of 15 of group B, and 0 of 11 of group C patients ($P = 0.018$). This observation is interesting with respect to recent reports linking lenalidomide to secondary malignancies in MM [4, 5]. The sample size in our analysis is small to derive definite conclusions.

Cytotoxic therapies cause BM damage by formation of highly mutagenic methylated-base-lesions, inter- and intrastrand crosslinks in DNA, DNA double strand-breaks, and impaired DNA repair [21–23]. Progressive accumulation of DNA-damaging mutations results in emergence of abnormal clones with a proliferative and survival advantage [21–23]. Li et al. [24] found differences in expression levels of genes related to mitochondrial function, metabolism, DNA repair, and cell cycle regulation in CD34+ hematopoietic stem and progenitor cells between patients with Hodgkin or non-Hodgkin lymphoma, who developed t-MDS/AML following autologous HSCT, and those (matched controls) who did not have such an outcome. Barletta et al. [25] reported detection of *RAS* mutations in the peripheral blood of patients with chemotherapy exposure, prior to onset of hematological abnormalities (cytopenia). These studies provide evidence that cytotoxic exposure makes the cells genetically unstable and vulnerable to subsequent insults. The exact host factors, or the type of, or dosage of chemo- or radiotherapy that can tip the balance and trigger development of t-MN are poorly understood, and may also involve the BM microenvironment [25]. Prior studies by Bejar et al. [26] and Thol et al. [27] demonstrated that point mutations in *TP53*, *EZH2*, *ETV6*, *RUNX1*, ASXL1, [26] and in genes involved in the splicing machinery [27] are significantly associated with progression from MDS to AML. By sequencing of

MDS genomes, scientists have hypothesized that early driver mutations, typically involving RNA splicing, direct evolution of the disease, and also influence the clinical phenotype including BM morphological features, blast count, hemoglobin level, and clinical presentation [26–29]. Although mutational analysis for these genes was not performed in this study, it is very likely that one or more such genetic events, not detected at the resolution level of metaphase cytogenetics or FISH were the trigger for leukemic transformation in these patients.

Conclusion

The FOAL in the index group of heavily pretreated patients suggests that hematopoietic dysregulation leading to this clinical event may be the result of genetic alterations at the DNA level that cannot be detected by conventional cytogenetics or FISH. Studies in our institution are in progress to determine whether MM- and stroma-related genetic features can be identified as contributors to clinical t-AL development. Our group is also working on a GEP-based model, which will help in identifying patients at increased risk of developing t-MDS after chemotherapy. We, and others [25, 30] envision that data from studies based on GEP and massively parallel, whole-genome sequence analysis will help to select specific, frequently occurring genomic aberrations that can reliably predict a t-MN event, and be used to develop targeted genetic tests for surveillance of patients receiving cytotoxic therapy. A collaborative approach to develop cost-effective clinical tests for submicroscopic genomic aberrations in this target population is clearly warranted.

Conflict of Interest

None declared.

Ethics

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

References

1. Godley, L. A., and R. A. Larson. 2008. Therapy-related myeloid leukemia. Semin. Oncol. 35:418–429.
2. Larson, R. A. 2009. Therapy-related myeloid neoplasms. Haematologica 94:454–459.
3. Leone, G., L. Fianchi, L. Pagano, and M. T. Voso. 2010. Incidence and susceptibility to therapy-related myeloid neoplasms. Chem. Biol. Interact. 184:39–45.
4. Attal, M., V. C. Lauwers, G. Marit, D. Caillot, T. Facon, C. Hulin, et al. 2010. Maintenance treatment with lenalidomide after transplantation for MYELOMA: final analysis of the IFM 2005-02. Blood (ASH Annu. Meet. Abstr.) 116:310A.
5. Usmani, S. Z., J. Sawyer, A. Rosenthal, M. Cottler-Fox, J. Epstein, S. Yaccoby, et al. 2013. Risk factors for MDS and acute leukemia following total therapy 2 and 3 for multiple myeloma. Blood 121:4753–4757.
6. Mailankody, S., R. M. Pfeiffer, S. Y. Kristinsson, N. Korde, M. Bjorkohlm, I. Turesson, et al. 2011. Risk of acute myeloid leukemia and myelodysplastic syndromes after multiple myeloma and its precursor disease (MGUS). Blood 118:4086–4092.
7. Govindarajan, R., S. Jagannath, J. T. Flick, D. H. Vesole, J. Sawyer, B. Barlogie, G. Tricot. Preceding standard therapy is the likely cause of MDS after autotransplants for multiple myeloma. 1996. Br. J. Haematol. 95:349–353.
8. Barlogie, B., G. Tricot, J. Haessler, F. van Rhee, M. Cottler-Fox, E. Anaissie, et al. 2008. Cytogenetically defined myelodysplasia after melphalan-based autotransplantation for multiple myeloma linked to poor hematopoietic stem-cell mobilization: the Arkansas experience in more than 3000 patients treated since 1989. Blood 111:94–100.
9. Barlogie, B., G. Tricot, E. Anaissie, J. Shaughnessy, E. Rassmusen, F. van Rhee, et al. 2006. Thalidomide and hematopoietic-cell transplantation for multiple myeloma. N. Engl. J. Med. 354:1021–1030.
10. Barlogie, B., E. Anaissie, F. van Rhee, J. Haessler, K. Hollmig, M. Pineda-Roman, et al. 2007. Incorporating bortezomib into upfront treatment for multiple myeloma: early results of total therapy 3. Br. J. Haematol. 138:176–185.
11. Nair, B., F. van Rhee, J. D. Shaughnessy Jr., E. Anaissie, J. Szymonifka, A. Hoering, et al. 2010. Superior results of Total Therapy 3 (2003-33) in gene expression profiling-defined low-risk multiple myeloma confirmed in subsequent trial 2006-66 with VRD maintenance. Blood 115:4168–4173.
12. Anaissie, E. J., F. van Rhee, A. Hoering, S. Waheed, Y. Alsayed, N. Petty, et al. 2010. Comparing toxicities and survival outcomes with total therapy 4 (TT4) for 70-gene (R70)-defined low-risk multiple myeloma (MM) to results obtained with total therapy 3 protocols TT3A and TT3B. Blood (ASH Annu. Meet. Abstr.) 116:368A.
13. Schanz, J., H. Tüchler, F. Solé, M. Malo, E. Luño, J. Cervera, et al. 2012. New comprehensive cytogenetic scoring system for primary myelodysplastic syndromes (MDS) and oligoblastic acute myeloid leukemia after MDS

derived from an international database merge. J. Clin. Oncol. 30:820–829.
14. Kyle, R. A., and S. V. Rajkumar. 2009. Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma: criteria for assessment of multiple myeloma. Leukemia 23:3–9.
15. Goasguen, J. E., and J. M. Bennett. 1992. Classification and morphologic features of the myelodysplastic syndromes. Semin. Oncol. 19:4–13.
16. Orazi, A., and U. Germing. 2008. The myelodysplastic/myeloproliferative neoplasms: myeloproliferative diseases with dysplastic features. Leukemia 22:1308–1319. doi:10.1038/leu.2008.119
17. Shaffer, L. G., M. L. Slovak, and L. J. Campbell, eds. 2009. ISCN 2009: an International System for Human Cytogenetic Nomenclature (2009): recommendations of the International Standing Committee on Human Cytogenetic Nomenclature. 1st ed. S. Karger AG, Switzerland.
18. Zhan, F., E. Tian, K. Bumm, R. Smith, B. Barlogie, J. Shaughnessy Jr. 2003. Gene expression profiling of human plasma cell differentiation and classification of multiple myeloma based on similarities to distinct stages of late-stage B-cell development. Blood 101:1128–1140. doi:10.1182/blood-2002-06-1737.
19. Zhan, F., Y. Huang, S. Colla, J. P. Stewart, I. Hanamura, S. Gupta, et al. 2006. The molecular classification of multiple myeloma. Blood 108:2020–2028.
20. Pemmaraju, N., D. Shah, H. Kantarjian, R. Z. Orlowski, G. M. Nogueras González, V. Baladandayuthapani, et al. 2014. Characteristics and outcome of patients with multiple myeloma who develop therapy-related myelodysplastic syndrome, chronic myelomonocytic leukemia, or acute myeloid leukemia. Clin. Lymphoma Myeloma Leuk. 2014 Jul 15. pii: S2152-2650(14)00261-4. doi: 10.1016/j.clml.2014.07.001. [Epub ahead of print].
21. Sill, H., W. Olipitz, A. Zebisch, E. Schulz, and A. Wolfler. 2011. Therapy-related myeloid neoplasms: pathobiology and clinical characteristics. Br. J. Pharmacol. 162:792–805.
22. Joannides, M., and D. Grimwade. 2010. Molecular biology of therapy-related leukaemias. Clin. Transl. Oncol. 12:8–14.
23. Pedersen-Bjergaard, J., M. K. Andersen, M. T. Andersen, and D. H. Christiansen. 2008. Genetics of therapy-related myelodysplasia and acute myeloid leukemia. Leukemia 22:240–248.
24. Li, L., M. Li, C. Sun, L. Francisco, S. Chakraborty, M. Sabado. 2011. Altered hematopoietic cell gene expression precedes development of therapy-related myelodysplasia/acute myeloid leukemia and identifies patients at risk. Cancer Cell 20:591–605.
25. Barletta, E., G. Gorini, P. Vineis, L. Miligi, L. Davico, G. Mugnai, et al. 2004. *RAS* gene mutations in patients with acute myeloid leukemia and exposure to chemical agents. Carcinogenesis 25:749–755.
26. Bejar, R., R. Levine, and B. L. Ebert. 2011. Unraveling the molecular pathophysiology of myelodysplastic syndromes. J. Clin. Oncol. 29:504–515.
27. Bejar, R., K. Stevenson, O. Abdel-Wahab, N. Galili, B. Nilsson, G. Garcia-Manero, et al. 2011. Clinical effect of point mutations in myelodysplastic syndromes. N. Engl. J. Med. 364:2496–2506.
28. Thol, F., S. Kade, C. Schlarmann, Löffeld, P., M. Morgan, and J. Krauter, et al. 2012. Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndromes. Blood 119:3578–3584.
29. Papaemmanili, M., L. Gerstung, L. Malcovati, S. Tauro, G. Gundem, P. Van Loo, et al. 2013. Clinical and biological implications of driver mutations in myelodysplastic syndrome. Blood 122:3616–3627.
30. Haferlach, T., Y. Nagata, V. Grossmann, Y. Okuno, U. Bacher, G. Nagae, et al. 2014. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia 28:241–247.