Leukemia Research 38 (2014) 756–763

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Lenalidomide and metronomic melphalan for CMML and higher risk MDS: A phase 2 clinical study with biomarkers of angiogenesis

Rena Buckstein[a,*], Robert Kerbel[b], Matthew Cheung[a], Yuval Shaked[c], Lisa Chodirker[a], Christina R. Lee[b], Martha Lenis[d], Cindy Davidson[a], Mary-Anne Cussen[d], Marciano Reis[e], Alden Chesney[e], Liying Zhang[d], Alexandre Mamedov[d], Richard A. Wells[a,f]

[a] Department of Medical Oncology/Hematology, Odette Cancer and Sunnybrook Health Sciences Center, Toronto, Canada
[b] Department of Medical Biophysics and Platform Biological Sciences, Sunnybrook Research Institute, Toronto, Canada
[c] Department of Molecular Pharmacology, Rappaport Faculty of Medicine Technion – Israel Institute of Technology, Haifa, Israel
[d] Department of Clinical Trials, Odette Cancer and Sunnybrook Health Sciences Center, Toronto, Canada
[e] Departments of Pathology and Laboratory Medicine, Sunnybrook Health Sciences Center, Toronto, Canada
[f] Department of Biological Sciences, Sunnybrook Research Institute, Toronto, Canada

ARTICLE INFO

Article history:
Received 9 January 2014
Received in revised form 2 March 2014
Accepted 28 March 2014
Available online 5 April 2014

Keywords:
Angiogenesis
MDS
CMML
Biomarker
Circulating endothelial cells
VEGF

ABSTRACT

Metronomic, low dose chemotherapy may have anti-angiogenic effects and augment the effects of lenalidomide in MDS and CMML. We evaluated the clinical efficacy, tolerability and anti-angiogenic effects of melphalan 2 mg and lenalidomide 10 mg for 21 days/28 in CMML ($n = 12$) and higher risk MDS ($n = 8$) patients in a prospective phase II study. The primary endpoint was overall response and secondary endpoints included survival, progression-free survival, toxicity and biomarkers of angiogenesis. The median age was 73 years, 55% were pretreated and transfusion dependent. The overall response rate was 3(15%) of 19 evaluable patients but 25% in CMML and 33% in pCMML. Dose reductions and/or delays were common due to myelosuppression. Transient spikes in circulating endothelial cells that declined below baseline were seen in responders and patients with CMML, suggesting anti-angiogenic activity. In conclusion, lenalidomide and metronomic low dose melphalan demonstrate signals of clinical and possible anti-angiogenic activity in patients with pCMML that require future validation. This trial was registered at clinicaltrial.gov under # NCT00744536.

1. Background

Myelodysplastic Syndromes (MDS) and chronic myelomonocytic leukemia (CMML) comprise a heterogeneous group of stem cell disorders resulting in ineffective hematopoiesis and clonal instability. The clinical presentation is typically that of cytopenias and functional cell defects and the development of acute myelogenous leukemia (AML) in approximately a third of patients [1]. Patients with CMML have monocytosis and may also have features of myeloproliferative neoplasms including splenomegaly and organ infiltration with monocytes. Patients with higher risk MDS as defined by the International Prognostic Scoring System (IPSS) [2] comprising 25–30% of cases, or higher risk CMML defined by the MD Anderson Scores [3,4] have shorter overall and leukemia-free survivals. Aberrantly increased angiogenesis in the bone marrow may be a factor contributing to disease progression. Bone marrow microvessel density increases as the blast percentage increases [5] and this increase is associated with inferior survival. Vascular endothelial growth factor (VEGF), the major positive regulator of angiogenesis, is present at increased concentrations in MDS blood and marrow [5,6]. Recently angiopoietin 1 (*Ang*-1) overexpression was found to be associated with higher risk and higher blast percentage MDS and to correlate with the development of AML. Furthermore, high levels of *Ang*-1 were predictive of shorter overall survival independent of karyotype, IPSS and age [7]. It has been hypothesized that these pro-angiogenic cytokines may promote leukemia cell propagation and survival via both paracrine and autocrine signaling [8]. Angiogenic factors may interact with other mechanisms, including differentiation and apoptosis in CMML and MDS [9,10]. By contributing to self- renewal of leukemia and MDS progenitors and elaboration of inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), it is logical to speculate that therapeutic targeting of angiogenesis may improve hematopoiesis,

* Corresponding author at: Odette Cancer Center, 2075 Bayview Avenue, Toronto, ON M4N3M5, Canada. Tel.: +1 416 480 5847; fax: +1 416 480 6002.
E-mail address: rena.buckstein@sunnybrook.ca (R. Buckstein).

http://dx.doi.org/10.1016/j.leukres.2014.03.022

delay the transition to leukemia and improve survival in these high-risk sub-groups.

There have been a limited number of largely negative clinical trials of anti-angiogenic therapies in MDS/AML or CMML, possibly because the agents were tested as monotherapies in highly advanced disease. In a German, French and Belgian (GFM) clinical trial, bevacizumab in conventional dosing was tested in MDS patients with excess blasts. Although the drug had pharmacodynamic activity as reflected by reduced VEGF and bone marrow microvessel density, only 1/20 patients had a significant hematologic response [11]. Minimal clinical activity was seen in another phase 2 trial of SU5416, a first generation small molecule VEGF receptor inhibitor for patients with refractory myeloproliferative diseases although CMML comprised only 18% of the total population [12].

Low-dose, metronomic chemotherapy (LDMC) is a strategy for optimizing the effects of chemotherapeutics by administering traditional cytotoxic drugs (*e.g.* melphalan, cyclophosphamide, vinblastine, etc.) at lower doses without prolonged rest periods. LDMC is thought to inhibit tumor angiogenesis through multiple possible mechanisms – by direct killing of activated endothelial cells in the tumor neovasculature [13,14], inhibition of tumor cell HIF-1α expression and by inhibition of circulating endothelial precursor cells [15]. Other possible non-angiogenic mechanisms of activity under investigation include immunomodulation (with the depletion of T regulatory cells and the upregulation of cytotoxic T cells) and cancer stem cell targeting [16,17]. Studies in animal models of tumors [13,14,18] have demonstrated that maximal inhibition of angiogenesis and tumor regression occurs when LDMC is combined with anti-angiogeneic drugs targeting the VEGF pathway. Recent phase II and III clinical trial results provide further support for their treatment combination [19,20].

Lenalidomide has clinical activity in non del5q MDS [21] and one mechanism of action may include its demonstrated anti-angiogenic activity [22] through the inhibition of VEGF and TNF-α induced endothelial cell migration and Akt phosphorylation [23].

Continuous oral low dose melphalan has demonstrated activity and safety in higher risk MDS and AML [24,25] particularly in hypocellular variants.

This phase II study explored the efficacy and safety of combination therapy with both lenalidomide and low dose continuous melphalan in patients with CMML or higher risk MDS and examined the effects on biomarkers of angiogenesis.

2. Patients and methods

2.1. Study design

This was a single-arm, single center phase 2 prospective trial of combination therapy with lenalidomide and melphalan in patients with CMML 1–2 or higher risk (int-2 or high) MDS defined by the IPSS [2] or transfusion dependent MDS with blast % ≥5. Patients had to demonstrate adequate hepatic and renal functions and performance status (ECOG ≤2). In patients with proliferative CMML-1 or 2 (pCMML, WBC > 12×10^9/L), risk group was assigned using an MD Anderson CMML prognostic scoring system [3]. Patients could receive up to 12 cycles of lenalidomide 10 mg po combined with melphalan 2 mg po daily for 21 days out of 28. Two dose reductions of lenalidomide and melphalan were permitted (see online Appendix A).

Patients remained on study drugs until progression, death, unacceptable adverse event or 12 months. Patients were followed until progression, death or data lock (March 2013). All patients provided informed consent.

2.2. Assessment of efficacy

The primary endpoint was overall response rate as defined by modified international working group standardized response criteria which included complete remission (CR), partial remission (PR), marrow CR (mCR) and hematologic improvement (HI) [26]. The secondary endpoints were cytogenetic remission rates, safety, OS, PFS, and biomarkers of angiogenesis including circulating endothelial cells (CECs) and precursors (CEPs), plasma VEGF and VEGFR 1–2 levels.

Bone marrows for response assessments were performed days 1 of cycles 3, 5, 8 and 11.

2.3. Adverse event assessment

Adverse events were identified and graded 1–5 using Common Toxicity Criteria v. 3.0. Hematologic toxicities were recorded throughout the study, and are reported as median percentage declines in absolute counts and increases in grade from baseline occurring during the first 12 weeks of therapy.

2.4. Evaluation of angiogenesis

Soluble plasma VEGF, VEGFR1 and 2 levels were measured at baseline, day 1 of cycles 1–3 and twice thereafter at intervals of 3 months, and at study discontinuation. CECs and CEPs were measured at the same time points and enumerated as previously described [27,28] (see online Appendix A).

2.5. Sample size and statistical analysis

The sample size for this study was based on Simon's optimal two-stage design (alpha = 0.05 and power = 80%) with 9 patients enrolled in the first stage. The trial would be terminated if 0 responses were observed during the first stage. Otherwise, an additional 8 patients would be accrued in the second stage. The regimen would be active if 2 or more responses were observed from 17 patients accrued. A total of 20 patients were planned to accommodate for an anticipated 20% dropout rate. Data were presented descriptively using median, interquartiles (IQR), and ranges for continuous variables; using proportions for categorical findings. Overall Survival (OS) and Progression-free Survival (PFS) were presented using Kaplan–Meier curves and log-rank test. To compare baseline biomarkers between groups of patients, non-nonparametric median test was conducted. To assess for correlations between selected biomarkers or blood counts, Spearman correlation coefficients were calculated with p-value < 0.05 considered statistically significant. All results were conducted using Statistical Analysis Software (SAS version 9.3 for Windows) package.

3. Results

The study received local Institutional Review Board approval and accrued between January 2008 and March 2011. This trial was registered at http://www.clinicaltrials.gov/ as NCT00744536. Twenty patients with histologically confirmed CMML or higher risk MDS were enrolled at the Odette Cancer Center for this study (Table 1). A preponderance of patients enrolled in this study had CMML (n = 12, 60%) and 9/12 had a total leukocyte count greater than 12×10^9/L. The median age was 73 years (range 52–87) and the median time from MDS diagnosis was 5.9 months (range 0.4–55). The median blast percentage was 6% (1–18) and 55% were transfusion dependent (TD) at enrollment. Thirty five percent had received a prior erythropoietic stimulating agent (ESA), 30% prior hydroxyurea and 19% prior valproic acid. Of those classifiable by the IPSS, 58% had Int-2 and 42% had high-risk disease. For the 12 CMML patients, the MD Anderson CMML prognostic scores were primarily intermediate-1 (42%) and Int-2 (50%) [3]. Sixty seven percent of the 9 pCMML patients (WBC > 12×10^6/l) had Int-2 risk scores. Patients received a median of 4 cycles (range 1–12) with a median cycle length of 30 days (range 20–63). The median follow up was 8 months (range (1–46) and median time on study was 5 months (range 1–12).

3.1. Response

19/20 patients were evaluable for response assessment (Table 2). One patient achieved a marrow CR at 68 days, 1 hematologic improvement (HI)-PLT at 63 days and 1 HI-erythroid at 147d for a total response rate of 15%. All three responses were seen in patients with proliferative CMML-1 (median number of cycles = 9, IQR 4–9). Nine patients (47%) had stable disease (median # cycles 6, IQR 4–9). Four patients (21%) were graded as failures due to worsening cytopenias without progression or death on study (median number of cycles = 2.5, IQR 1–4) and 3 patients (16%) had progressive disease (median number of cycles = 3, IQR 3–3). The 9 patients with pCMML had a median WBC of 46×10^9/L (range 19–312) at baseline that declined by 82% over the first 3 cycles. Similarly, the LDH, which was elevated at baseline (313 IU/L), declined by 37% to

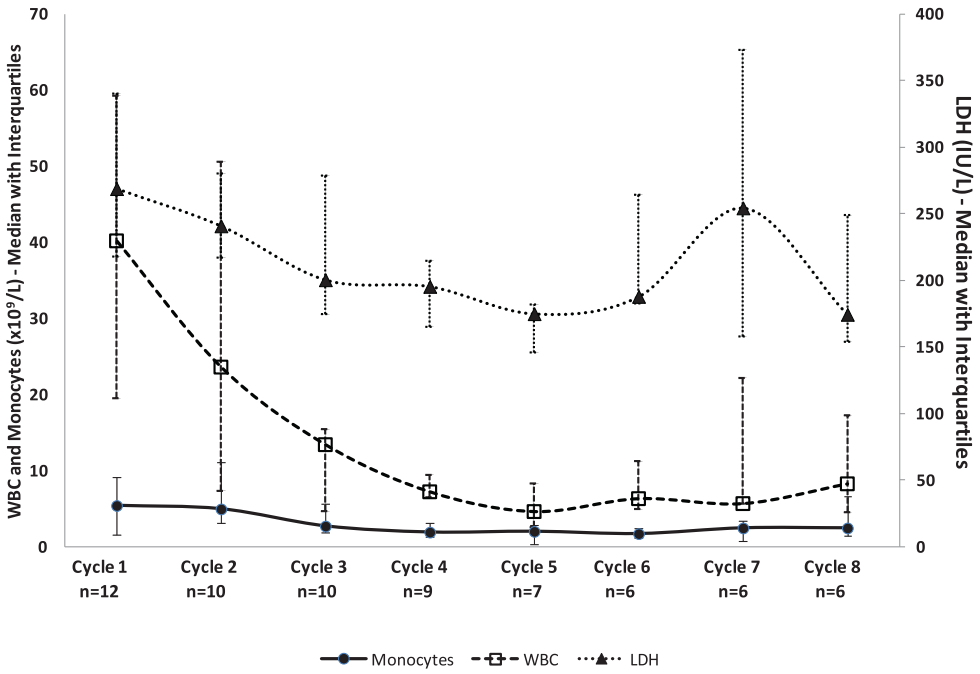

Fig. 1. WBC: White blood cells × 10^9/L. LDH: Lactate dehydrogenase (IU/L). IQR: Interqurtile range.

a normal level (196 IU/L) after 3 cycles (Fig. 1). One notable patient with pCMML-1 who had initially presented with rapidly increasing leukocytosis, red cell and platelet transfusion dependence, symptomatic pleural effusions and peripheral edema had resolution of her effusions and edema by cycle 4 and became red cell transfusion independent by 147 days. She remained alive, effusion/edema free with a normal WBC and LDH at a follow up of 3.9 years from enrollment. She remained off all therapy, red cell and platelet transfusion independent despite only receiving 9 months of treatment. In contrast, another patient with pre-treated pCMML-1 had a dramatic decrease in his WBC (from 312 to 78 × 10^9/L) and LDH (from 900 to 450 IU/L) after 1 cycle of therapy but remained persistently symptomatic with unremitting ascites and peripheral edema that worsened markedly on the 1-week drug holiday. As a result, he was taken off study after 3 cycles, despite achieving a HI-P.

Only 1 patient with stable disease completed all 12 cycles and 1 patient became red cell transfusion independent on study. The reasons for premature discontinuation included death ($n = 3$), progressive disease ($n = 5$) and toxicity ($n = 11$).

3.2. Survival

In total, 5 progressed and three died on study. At follow-up, 19 patients have died: 10 due to progressive disease and 9 from other causes (myocardial infarction ($n = 1$), congestive heart failure ($n = 3$), pneumonia ($n = 2$), post hip surgery complication ($n = 1$), liver failure ($n = 1$) and unknown ($n = 1$). The median overall and progression-free survivals were 8.5 months (95% CI 5.9–12.7) and 7.7 months (95% CI 4.2–12.3) respectively and are depicted in Fig. 2.

3.3. Assessment of safety

Despite a high rate of baseline cytopenias (Table 2B) worsening hematologic toxicity was prevalent. Within the first 12 weeks of therapy, 20% grade 3 and 45% grade 4 neutropenia, 10% grade 3 and 75% grade 4 thrombocytopenia were documented (Table 2C). While day 1 cell counts for subsequent cycles often improved compared with nadirs, 45% and 60% had grades 3–4 neutropenia and thrombocytopenia respectively at these time points. This represented a worsening in grade neutropenia and thrombocytopenia (compared with baseline) for 40% and 55% of patients. As a result, dose delays and/or reductions were common. Of the 16 patients that had 2 or more cycles, 9/16 had a lenalidomide dose reduction by or during cycle 3:6/9 to 5 mg daily and 3/9 to 5 mg alternate days. In two additional patients dosage was reduced to 5 mg daily after cycle 4. Similarly, 8/16 patients had a melphalan dose reduction to 2 mg alternate days by or during cycle 3, and by cycle 5, 4/8 were dose reduced, 2 to 2 mg alternate days and 2 to 2 mg twice weekly. Four patients discontinued therapy within the first cycle for toxicity or disease progression and 7 patients by cycle 3. Three patients died on study, 1 from transfusion associated circulatory overload (TACO) post red cell transfusion in cycle 1, 1 from pneumonia in cycle 1 and 1 from progressive disease and blast crisis post cycle 9. No death was felt to be directly treatment related.

The grade 3–4 non-hematologic toxicities (and attributions) are summarized in Table 2A. The most common non-hematologic toxicities were infection (pneumonia ($n = 2$), febrile neutropenia ($n = 3$), cellulitis ($n = 1$)), neurologic (syncope $n = 2$, ischemic event $n = 1$), and cardiac (atrial fibrillation ($n = 1$), congestive heart failure ($n = 1$) and TACO ($n = 1$, grade 5). There was 1 episode of grade 3 epistaxis.

3.4. Biomarkers

Angiogenesis related biomarkers were available in 19 patients at baseline, 16 patients at cycle 2 day 1, 15 patients at cycle 3 day 1 with reducing numbers thereafter due to patient attrition (Fig. 3). CEPs were too infrequent to measure for most patients and are not reported. End of study (EOS) readings were available in 5 patients with either response or stable disease at a median time of 37 days (range 28–50 days) post last dose. When plotted over time for all patients, there were no discernable declines in serum VEGF or circulating VEGFR1 or VEGFR2 although an apparent transient spike in CECs was noted during the first cycle that then reverted to baseline or declined by cycle 6 (data not shown). Sample sizes are generally too small to detect statistically significant differences

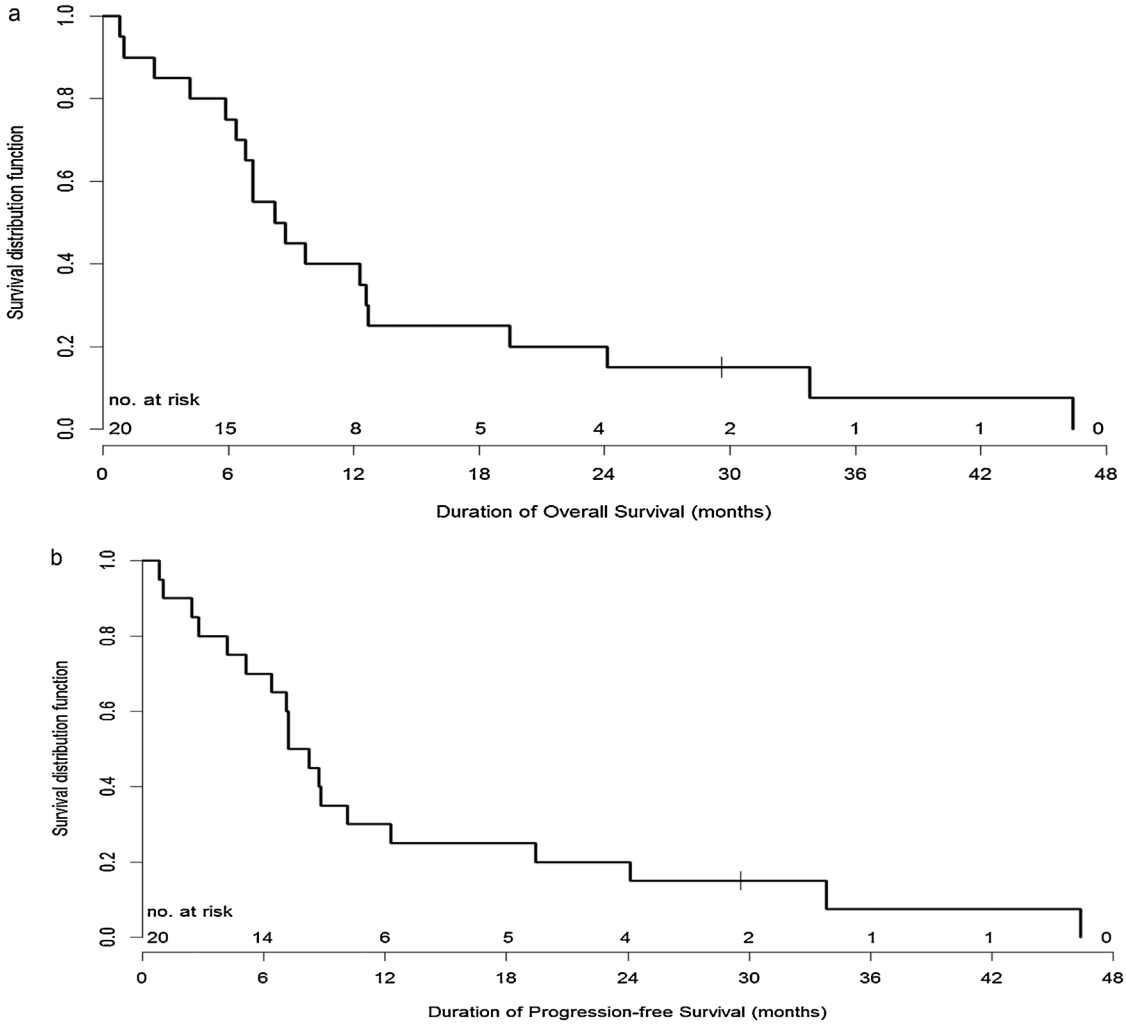

Fig. 2. (A) Overall survival. (B) Progression-free survival.

in the changes between responders ($n = 3$), stable disease ($n = 9$) and patients with progressive disease and failure ($n = 6$) but it is instructive to observe any trends according to response (Fig. 3a–c). Responding patients had higher levels of baseline CECs compared with non responders (10.35 cells/μl, IQR 6.35–187 versus 4.48 cells/μl, IQR 2.24–13.8, $p = .06$). Responders also appeared to have a 19 fold spike in total (primarily viable) CECs after the first cycle of therapy (from 10.35 cells/μl, IQR 6.3–187.2 to 193.7 cells/μl, IQR 18.9–268.4) before reverting back to baseline and declining in subsequent cycles. This compares with a 6 fold spike in total CECs after the first cycle in patients with progressive disease or failure (from 3.8 cells/μl, IQR 3.4–4.4 to 23.9 cells/μl, IQR 1–46). Similarly, in CMML we also documented transient spikes in total CECs after cycle 1 (from 6.67 cells/μl, IQR 3.7–16.2 to 24 cells/μl, IQR 5.2 to 50) that gradually declined by 86% at cycle 6, only to increase again toward the end of study drugs (Fig. 3d–e).

The median baseline VEGF levels were higher for CMML than for non-CMML patients (43.45 versus 26.5 pg/ml) but they were not statistically different ($p = 0.22$). Baseline CECs were significantly higher in the CMML patients compared with the other WHO MDS subtypes (6.67 versus 3.45 cells/μl, $p = .03$), (Table 3). In the CMML patients, VEGF, sVEGFR1, sVEGFR2 and CEC were not significantly correlated with the monocyte count, total WBC or LDH.

CECs were weakly correlated with sVEGFR1 at baseline ($r = 0.45$, $p = .05$) and with serum VEGF on day 1 cycle 2 ($r = 0.68$, $p = .02$).

4. Discussion

Metronomic chemotherapy is under active investigation in the clinic, especially for the treatment of solid tumors. As recently reviewed, there are more than 80 published phase 2–3 studies encompassing 3688 patients with a variety of cancers that have demonstrated the clinical benefits of LDMC. The most commonly used drugs are cyclophosphamide, and oral 5-FU (5 fluorouracil) pro-drugs capecitabine or UFT (tegafur-uracil). LDMC is frequently combined with other therapies (64%) [29]. Anti-angiogenic therapy in general and LDMC specifically have been infrequently evaluated in blood cancers but show promise in the lymphoid neoplasms, particularly with the drug cyclophosphamide [30–32]. The LDMC/anti-angiogenic field was recently bolstered by the positive results of the CAIRO3 – a clinical trial of the Dutch Colorectal Cancer Group. CAIRO3 demonstrated a significant PFS benefit to maintenance treatment with bevacizumab + oral daily low dose capecitabine in patients with metastatic colorectal carcinoma [20].

Before azacitidine was available there were limited therapeutic options for patients with CMML and higher risk MDS. Building on our local experience with metronomic cyclophosphamide and celecoxib in relapsed or refractory aggressive lymphomas [31] we chose to evaluate the efficacy, safety and anti-angiogenic activity of metronomic melphalan and lenalidomide in CMML and MDS patients. During the course of the study, azacitidine became

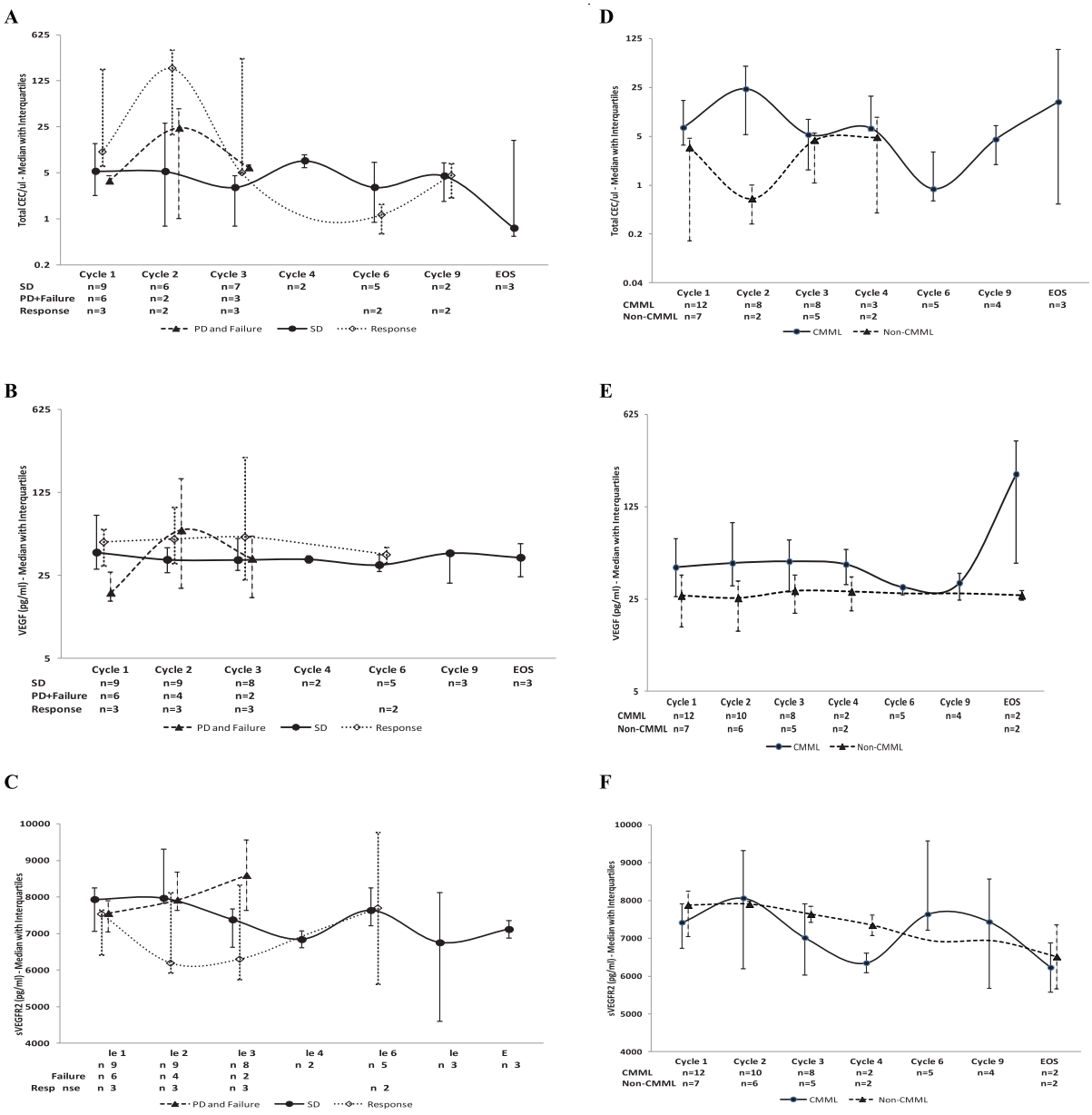

Fig. 3. (A) CEC according to response. (B) VEGF according to response. (C) VEGFR2 according to response. (D) CEC according to CMML or not. (E) VEGF according to CMML or not. (F) VEGFR2 according to CMML or not. CEC: Circulating endothelial cells. VEGF: Vascular endothelial cell growth factor. VEGFR2: Vascular endothelial growth factor receptor-2. SD: Stable disease. PD: Progressive disease.

available for int-2 and high risk MDS and explains the predominance of CMML patients on this study.

To our knowledge, this is the first clinical trial to evaluate LDMC as possible anti-angiogenic therapy in CMML and MDS. We chose the immunomodulatory drug lenalidomide because it is modestly active as monotherapy in non-del5q MDS [21] and in higher risk del5q MDS [33] even after azacitidine failure [34]. One mechanism of action in non-del5q MDS may include the inhibition of angiogenesis [22,23]. We chose to partner lenalidomide with low continuous dose melphalan because of its demonstrated activity and safety in higher risk MDS and AML [24,25]. We hypothesized that when used in such a metronomic schedule this drug may be functioning in part via anti-angiogenic mechanisms.

In this study, we found a modest overall response rate of 15%, and a response rate of 25% in CMML overall (3/12) and 33% in proliferative CMML (3/9). Our patients experienced significant myelosuppression at our starting doses of melphalan 2 mg od and lenalidomide 10 mg od for 21d/28 schedule. This necessitated drug reductions and dose delays in the majority of the patients within 2 cycles and 5 patients came off study early for thrombocytopenia. While four patients discontinued treatment within 1 treatment cycle due to death, disease progression or severe pancytopenia, it is often difficult to ascribe worsening blood counts to drug toxicity or natural history in advanced pre-treated MDS and CMML. Our experience is consistent with that observed in higher risk del5q MDS where lenalidomide monotherapy was also associated with significant myelosuppression and adverse events necessitating dose reductions to 5 mg/day in 72% and discontinuation after only 1 cycle in 31% [33].

We chose to include CMML in our patient population because there were limited therapeutic options at the time. CMML is now a distinct entity defined in the WHO as an MDS/MPN, not strictly MDS. It is classified as CMML-1 or 2 according to blast percentage in the bone marrow and peripheral blood and has its own clinical

Table 1
Baseline characteristics $n = 20$.

Baseline characteristics	N (%)	
Age at consent (year)		
Median (range)		73 (52–87)
Gender		
Male/female	13/7	(65%/35%)
WHO subtype		
RCMD-RS	1	(5.00%)
RAEB-1	2	(10.00%)
RAEB-2	5	(25.00%)
CMML-1	10	(50.00%)
CMML-2	2	(10.00%)
IPSS risk group ($n = 11$ evaluable, includes 3 CMML with low WBC)		
Int-2 MDS	9	(82.00%)
High risk MDS	2	(18.00%)
IPSS – MD Anderson CMML ($n = 12$)		
Low	1	(8.33%)
Int-1	5	(41.67%)
Int-2	6	(50.00%)
Time from diagnosis (months)		
Median (range)		5.9 (0.4–55.1)
Hgb		
Median (range)		93.5 (68.0–122.0)
ANC (absolute neutrophils count)		
Median (range)		5.2 (0.3–124.7)
Platelets		
Median (range)		56 (7–621)
Transfusion dependence at baseline		
TI	9	(45.00%)
TD	11	(55.00%)
Previous therapy ($n = 12$)		
ESA	7	(58.33%)
Lenalidomide	0	(0%)
Immunosuppressive therapy	1	(8.33%)
Hydroxyurea	6	(50.00%)
Valproic acid	2	(16.67%)
Bone marrow blast (%)		
Median (range)		6 (1–18)
Karyotype		
Good risk	12	(66.6%)
Intermediate risk	2	(11.11%)
Poor risk	4	(22.2%)

WHO: World health organization, IPSS: International prognostic scoring system, Hgb: Hemoglobin, TI: Transfusion independent, TD: Transfusion dependent.

Table 2
Adverse events.

2A Non-hematologic toxicities	Grades 3–4 on study	
	n (%)	Possible/probably/definitely related to protocol therapy n (%)
Infection	6 (30%)	6 (30%)
Neurological	3 (15%)	3 (15%)
Cardiac (CHF, arrhythmia)	3 (15%)	0 (0%)
Endocrine	1 (5%)	1 (5%)
Hemorrhage/bleeding	1 (5%)	1 (5%)
Pain	1 (5%)	1 (5%)
Constitutional symptoms	1 (5%)	0 (0%)
Gastrointestinal	1 (5%)	0 (0%)
Musculoskeletal/soft/tissue	1 (5%)	0 (0%)
Pulmonary	1 (5%)	0 (0%)

2B	Hematologic toxicity grades@ baseline ($n = 20$)			
	1	2	3	4
Anemia	7 (35%)	9 (45%)	3 (15%)	0 (0%)
Leukocytes	1 (5%)	2 (10%)	3 (15%)	0 (0%)
Neutrophils	2 (10%)	1 (5%)	2 (10%)	3 (15%)
Platelets	6 (30%)	3 (15%)	6 (30%)	2 (10%)

2C	Hematologic toxicity grades on study ($n = 20$) (first 12 weeks)			
	1	2	3	4
Anemia	4 (20%)	5 (25%)	8 (40%)	3 (15%)
Leukocytes	3 (15%)	3 (15%)	5 (25%)	4 (20%)
Neutrophils	0 (0%)	3 (15%)	4 (20%)	9 (45%)
Platelets	1 (5%)	2 (10%)	2 (10%)	15 (75%)

Grade 3–4 non hematologic toxicities on study and relatedness to therapy. 2B: All grade hematologic toxicities at baseline. 2C: All grade hematologic toxicities on study during first 12 weeks.

prognostic scoring systems, with most systems reporting inferior survival for proliferative forms defined by a WBC > 12×10^9/L [3,35,36,37]. There are a limited number of published case reports or clinical trials discussing the activity of lenalidomide in CMML as monotherapy [38] or in combination with bortezomib [39]. While the hypomethylating agents azacitidine and decitabine are approved for CMML based on activity observed in phase 2 and 3 trials, CMML (particularly pCMML) is often underrepresented in such trials [40–42]. Our study was conceived before the availability of HMA therapy for CMML in Ontario, which has subsequently become available but restricts reimbursement to patients with WBC < 13×10^9/L, based on AZA-001 inclusion criteria [40]. Similarly, the EMA (European Medical Agency) has restricted the approval of azacitidine for the treatment of CMML-2 in the absence of myeloproliferative aspects, which restricts access to only 10–20% of patients. This leaves hydroxyurea as the primary option for patients with proliferative CMML which is not always long-lasting nor effective. In addition, the OS after azacitidine failure is short [43] therefore alternative therapies are needed in these circumstances.

It is interesting to note that the 3 responding patients all had pCMML (out of 9 total, ORR 33%) and remained on drug for a median of 9 cycles suggesting a possible signal of activity and tolerability unique to this histology. The anti-proliferative effects of lenalidomide and melphalan in CMML are also evident in the declines seen in LDH, WBC and monocyte count (Fig. 1). The higher baseline levels of VEGF and CEC's in CMML patients compared with other MDS subtypes may point to higher levels of angiogenesis driving this disease as previously observed [6,44,45] and a potential 'druggable' target for appropriately selected patients.

Mature circulating endothelial cells (CECs) are in most cases apoptotic in healthy subjects and more viable in cancer patients, and represent an indirect marker of vessel damage and/or turnover and remodeling associated with angiogenesis [46]. CECs are significantly increased in different types of cancer [47] but because they are rare events, they need to be enumerated in dedicated laboratory with experienced personnel that use rigorous, robust and validated multiparametric procedures [48]. Changes in CECs and or CEP levels during therapy have been found to correlate with clinical outcomes of response and/or survival in 5 studies, but baseline levels have been inconsistently predictive of response in clinical trials of anti-angiogenic agents [49]. In one study, a CEC count of > 11uL after 2 months was predictive of improved overall and disease-free survival and the increase was attributed to an increased fraction of apoptotic CEC's [27]. In that study, a significant decline in VEGF-A levels after 2 months of therapy was associated with a significantly improved time to progression. In the current study, the increase in CEC values observed prior to cycle 2 in responding patients and those with CMML was primarily of *viable* cells. This is consistent with results from a preclinical model [50], in which a maximally tolerated dose (MTD) regimen caused short-term suppression of viable CECs and CEPs immediately after drug administration, followed by a robust rebound effect leading to an increased number of viable cells prior to the next cycle. The declines we observed

Table 3
Biomarkers at baseline (cycle 1 day 1) according to response or histology.

	N	CEC (IQR)	VEGF (IQR)	VEGFR1 (IQR)	VEGFR2 (IQR)
Response	3	10.35 (6.3–187)	47.7 (30.1–61.4)	125.0 (106.7–257.6)	7534 (6418.4–7640.9)
PD + Failure	6	3.7 (3.4–4.5)	17.96 (15.1–26.5)	100.2 (78–105.7)	7557.9 (7040–7895.8)
SD	9	5.3 (2.2–13.8)	39.9 (21.2–80.7)	104.6 (93.6–119.2)	7534 (6418.4–7640.9)
CMML	12	6.7 (3.7–16)	43.4 (25.9–71.6)	106.9 (100.2–127)	7418.9 (6736.1–7916.8)
MDS (non-CMML)	7	3.4 (0.2–4.65)	26.5 (15.4–37.8)	95.1 (78–105.7)	7875.2 (7040.7–8244.7)

PD: Progressive disease, SD: Stable disease, CMML: Chronic myelomonocytic leukemia, MDS: Myelodysplastic syndrome, CEC: Circulating endothelial cells/μl, IQR: Interquartile range, VEGF: Vascular endothelial growth factor (pg/ml), VEGFR-1: Vascular endothelial growth factor receptor 1(pg/ml), VEGFR-2: Vascular endothelial growth factor receptor 2(pg/ml).

in subsequent cycles in responding patients and those with CMML (who remained on drug long enough) may have reflected the almost ubiquitous dose reductions in lenalidomide and melphalan perhaps transforming lenalidomide and metronomic melphalan into anti-angiogenic rather than MTD cytotoxic agents. Higher levels of baseline CECs as biomarkers predictive of response to this regimen will need validation in more patients.

Numbers of viable CEC's and CEP's are increased in the peripheral blood of patients with advanced MDS and AML, and patients with higher levels of CECs also showed increased microvessel density, a surrogate marker of increased angiogenesis [51]. In a phase 1 study of ABT-751, a novel microtubule inhibitor, decreases in CEC numbers correlated with declines in WBC counts [52] although this was not our experience. Given that CEC are directly calculated based on the total WBC, this non-significant correlation supports the notion that any declines in CEC were related to other factors and not just non-specific myelosuppression.

Although early reports described an association between increased angiogenesis and higher risk MDS subtypes, recent findings have shown higher CEC levels in patients with *lower* risk MDS and have established negative correlations between CEC number and IPSS risk categories; similarily, microvessel density was most positively correlated with CEC number in patients with low-risk MDS [53]. Congruently, serum VEGFR2 was significantly higher in the lower IPSS risk classes [51]. It has been hypothesized that the need for angiogenic support to the outgrowth of neoplastic cells may be more important in earlier stages of the disease than in later stages in which other pathogenic factors may play a more significant role [51,53]. In light of this, the optimal histologic MDS subtypes in which to test the clinical efficacy of anti-angiogenic agents remains unclear but may be in fact lower risk disease.

The limitations of this study include the small overall and subgroup sample sizes and the fact that one cannot determine if the activity seen is primarily attributable to lenalidomide, melphalan or the combined approach. Nevertheless, in past pre-clinical [13,14,18] tumor models and clinical studies [54], combining 2 or 3 therapies with presumed 'anti-angiogenic' activity was needed for maximal therapeutic benefit. The combination of lenalidomide with azacitidine in MDS may be superior to azactidine monotherapy and is currently being tested in a controlled intergroup trial led by SWOG in higher risk MDS and CMML [55] (http://www.clinicaltrials.gov/: NCT01522976).

5. Conclusions

Metronomic low dose melphalan combined with lenalidomide is inactive in higher risk MDS and poorly tolerated. There is a signal of moderate clinical activity in patients with proliferative CMML (a form of MDS/MPN). While the mechanism of action may be primarily cytotoxic, the inhibition of angiogenesis cannot be excluded as a contributing factor. This requires further testing and validation in a larger more homogeneous population. For future studies, recommended starting doses would be lenalidomide 5 mg po daily and melphalan 2 mg po alternate days for 21 days/28. Easier to measure biomarkers are needed to identify appropriate candidates for such therapies.

Role of funding source

This investigator initiated study was funded by an unrestricted educational grant from Celgene. All data were maintained and analyzed by the principal investigator independently. No medical writers were used.

Conflicts of interest statement

RB and RW: Research funding from Celgene, honoraria for lectures and advisory board meetings. RK: Consultant to Taiho Pharmaceuticals and Cerulean Pharma, Lecture fees from Eli Lilly and Pfizer, research funding from Cerulean. All other authors: no COI to disclose.

Acknowledgements

We would like to acknowledge Dr. David Spaner for editorial and content suggestions.

Contributors: RB: Designed the study, accrued patients, analyzed results and assembled the manuscript. RSK and YS: Provided input into the design of the study and contributed to manuscript preparation. MC and RAW: Contributed patients and assisted with manuscript preparation. ML and AM: Assisted with data assembly, analysis and manuscript preparation. MAC and CD: Assisted with clinical trial operation and manuscript preparation. CL: Coordinated biomarker assays and summaries and assisted with manuscript preparation. MR and AC: Assisted with the study conduct and manuscript preparation. LZ: Provided biostatistical support and assisted with manuscript preparation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.leukres.2014.03.022.

References

[1] Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood 2002;100:2292–302.
[2] Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 1997;89:2079–88.
[3] Onida F, Kantarjian HM, Smith TL, Ball G, Keating MJ, Estey EH, et al. Prognostic factors and scoring systems in chronic myelomonocytic leukemia: a retrospective analysis of 213 patients. Blood 2002;99:840–9.
[4] Beran M, Wen S, Shen Y, Onida F, Jelinek J, Cortes J, et al. Prognostic factors and risk assessment in chronic myelomonocytic leukemia: validation study of the M.D. Anderson Prognostic Scoring System. Leuk Lymphoma 2007;48:1150–60.
[5] Aguayo A, Kantarjian H, Manshouri T, Gidel C, Estey E, Thomas D, et al. Angiogenesis in acute and chronic leukemias and myelodysplastic syndromes. Blood 2000;96:2240–5.

[6] Bellamy WT, Richter L, Sirjani D, Roxas C, Glinsmann-Gibson B, Frutiger Y, et al. Vascular endothelial cell growth factor is an autocrine promoter of abnormal localized immature myeloid precursors and leukemia progenitor formation in myelodysplastic syndromes. Blood 2001;97:1427–34.
[7] Cheng CL, Hou HA, Jhuang JY, Lin CW, Chen CY, Tang JL, et al. High bone marrow angiopoietin-1 expression is an independent poor prognostic factor for survival in patients with myelodysplastic syndromes. Br J Cancer 2011;105: 975–82.
[8] Dias S, Hattori K, Zhu Z, Heissig B, Choy M, Lane W, et al. Autocrine stimulation of VEGFR-2 activates human leukemic cell growth and migration. J Clin Invest 2000;106:511–21.
[9] Aguayo A. The role of angiogenesis in the biology and therapy of myelodysplastic syndromes. Curr Hematol Rep 2004;3:184–91.
[10] Raza A, Mundle S, Shetty V, Alvi S, Chopra H, Span L, et al. Novel insights into the biology of myelodysplastic syndromes: excessive apoptosis and the role of cytokines. Int J Hematol 1996;63:265–78.
[11] Legros L, Slama B, Karsenti JM, Vey N, Natarajan-Ame S, Watel E, et al. Treatment of myelodysplastic syndromes with excess of blasts by bevacizumab is well tolerated and is associated with a decrease of VEGF plasma level. Ann Hematol 2012;91:39–46.
[12] Giles FJ, Cooper MA, Silverman L, Karp JE, Lancet JE, Zangari M, et al. Phase II study of SU5416 – a small-molecule, vascular endothelial growth factor tyrosine-kinase receptor inhibitor – in patients with refractory myeloproliferative diseases. Cancer 2003;97:1920–8.
[13] Klement G, Baruchel S, Rak J, Man S, Clark K, Hicklin DJ, et al. Continuous low-dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity. J Clin Invest 2000;105: R15–24.
[14] Browder T, Butterfield CE, Kraling BM, et al. Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. Cancer Res 2000;60:1878–86.
[15] Shaked Y, Emmenegger U, Man S, Cervi D, Bertolini F, Ben-David Y, et al. The optimal biological dose of metronomic chemotherapy regimens is associated with maximum antiangiogenic activity. Blood 2005;106:3058–61.
[16] Loven D, Hasnis E, Bertolini F, Shaked Y. Low-dose metronomic chemotherapy: from past experience to new paradigms in the treatment of cancer. Drug Discov Today 2013;18:193–201.
[17] Nars MS, Kaneno R. Immunomodulatory effects of low dose chemotherapy and perspectives of its combination with immunotherapy. Int J Cancer 2013;132:2471–8.
[18] Hashimoto K, Man S, Xu P, Cruz-Munoz W, Tang T, Kumar R, et al. Potent preclinical impact of metronomic low-dose oral topotecan combined with the antiangiogenic drug pazopanib for the treatment of ovarian cancer. Mol Cancer Therapeut 2010;9:996–1006.
[19] Garcia AA, Hirte H, Fleming G, Yang D, Tsao-Wei DD, Roman L, et al. Phase II clinical trial of bevacizumab and low-dose metronomic oral cyclophosphamide in recurrent ovarian cancer: a trial of the California, Chicago, and Princess Margaret Hospital phase II consortia. J Clin Oncol 2008;26:76–82.
[20] Koopman M, Simkens L, Ten Tije A, Creemers G, Loosveld OJL, de Jongh FE. Maintenance treatment with capecitabine and bevacizumab versus observation after induction treatment with chemotherapy and bevacizumab in metastatic colorectal cancer (mCRC): the phase III CAIRO3 study of the Dutch Colorectal Cancer Group (DCCG). J Clin Oncol 2013;(Suppl.) (Abstract 3502).
[21] Raza A, Reeves JA, Feldman EJ, Dewald GW, Bennett JM, Deeg HJ, et al. Phase 2 study of lenalidomide in transfusion-dependent, low-risk, and intermediate-1 risk myelodysplastic syndromes with karyotypes other than deletion 5q. Blood 2008;111:86–93.
[22] Lu L, Payvandi F, Wu L, Zhang LH, Hariri RJ, Man HW, et al. The anti-cancer drug lenalidomide inhibits angiogenesis and metastasis via multiple inhibitory effects on endothelial cell function in normoxic and hypoxic conditions. Microvascular Res 2009;77:78–86.
[23] Dredge K, Horsfall R, Robinson SP, Zhang LH, Lu L, Tang Y, et al. Orally administered lenalidomide (CC-5013) is anti-angiogenic in vivo and inhibits endothelial cell migration and Akt phosphorylation in vitro. Microvascular Res 2005;69:56–63.
[24] Denzlinger C, Bowen D, Benz D, Gelly K, Brugger W, Kanz L. Low-dose melphalan induces favourable responses in elderly patients with high-risk myelodysplastic syndromes or secondary acute myeloid leukaemia. Br J Haematol 2000;108:93–5.
[25] Robak T, Szmigielska-Kaplon A, Urbanska-Rys H, Chojnowski K, Wrzesien-Kus A. Efficacy and toxicity of low-dose melphalan in myelodysplastic syndromes and acute myeloid leukemia with multilineage dysplasia. Neoplasma 2003;50:172–5.
[26] Cheson BD, Greenberg PL, Bennett JM, Lowenberg B, Wijermans PW, Nimer SD, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood 2006;108:419–25.
[27] Mancuso P, Colleoni M, Calleri A, Orlando L, Maisonneuve P, Pruneri G, et al. Circulating endothelial-cell kinetics and viability predict survival in breast cancer patients receiving metronomic chemotherapy. Blood 2006;108:452–9.
[28] Bertolini F, Shaked Y, Mancuso P, Kerbel RS. The multifaceted circulating endothelial cell in cancer: towards marker and target identification. Nat Rev Cancer 2006;6:835–45.
[29] Lien K, Georgsdottir S, Sivanathan L, Chan K, Emmenegger U. Low-dose metronomic chemotherapy: a systematic literature analysis. Eur J Cancer 2013;49:3387–95.
[30] Coleman M, Ruan G, Elstrom RL, Martin P, Leonard JP. Metronomic therapy for refractory/relapsed lymphoma: the PEP-C low-dose oral combination chemotherapy regimen. Hematology 2012;17(Suppl. 1):S90–2.
[31] Buckstein RJ, Crump M, Shaked Y, Foden C, Turner R, Taylor R, et al. High dose celecoxib and metronomic low-dose cyclophosphamide is effective and safe therapy in patients with relapsed and refractory aggressive histology NHL. Clin Cancer Res 2006;12:5190–8.
[32] Fassas ABT, Kiwan E, Roberts J, Burr T, Tricot G, Anaissie E, et al. Metronomic chemoimmunotherapy in a patient with refractory Waldenström's macroglobulinemia. Leuk Lymphoma 2005;46:1675–7.
[33] Ades L, Boehrer S, Prebet T, Beyne-Rauzy O, Legros L, Ravoet C, et al. Efficacy and safety of lenalidomide in intermediate-2 or high-risk myelodysplastic syndromes with 5q deletion: results of a phase 2 study. Blood 2009;113: 3947–52.
[34] Prebet T, Charbonnier A, Gelsi-Boyer V, Mozziconacci MJ, Blaise D, Vey N. Lenalidomide treatment for patients with myelodysplastic syndrome and low blast count acute myeloid leukemia after azacitidine failure. Leuk Lymphoma 2013;54:1538–40.
[35] Germing U, Kundgen A, Gattermann N. Risk assessment in chronic myelomonocytic leukemia (CMML). Leuk Lymphoma 2004;45:1311–8.
[36] Germing U, Strupp C, Knipp S, Kuendgen A, Giagounidis A, Hildebrandt B, et al. Chronic myelomonocytic leukemia in the light of the WHO proposals. Haematologica 2007;92:974–7.
[37] Such E, Germing U, Malcovati L, Cervera J, Kuendgen A, Della Porta MG, et al. Development and validation of a prognostic scoring system for patients with chronic myelomonocytic leukemia. Blood 2013;121:3005–15.
[38] Carus G. lenalidomide as induction therapy before allogeneic stem cell transplantation in a patient with proliferative CMML-2 and del(5q) not involving the EGR1 locus. Leukemia 2007;21:2384–5.
[39] Attar EC, Amrein PC, Fraser JW, Fathi AT, McAfee S, Wadleigh M, et al. Phase I dose escalation study of bortezomib in combination with lenalidomide in patients with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Leuk Res 2013;37:1016–20.
[40] Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol 2009;10:223–32.
[41] Lubbert M, Suciu S, Baila L, Ruter BH, Platzbecker U, Giagounidis A, et al. Low-dose decitabine versus best supportive care in elderly patients with intermediate- or high-risk myelodysplastic syndrome (MDS) ineligible for intensive chemotherapy: final results of the randomized phase III study of the European Organisation for Research and Treatment of Cancer Leukemia Group and the German MDS Study Group. J Clin Oncol 2011;29:1987–96.
[42] Wijermans PW, Ruter B, Baer MR, Slack JL, Saba HI, Lubbert M. Efficacy of decitabine in the treatment of patients with chronic myelomonocytic leukemia (CMML). Leuk Res 2008;32:587–91.
[43] Prebet T, Gore SD, Esterni B, Gardin C, Itzykson R, Thepot S, et al. Outcome of high-risk myelodysplastic syndrome after azacitidine treatment failure. J Clin Oncol 2011;29:3322–7.
[44] Cortes J. CMML: a biologically distinct myeloproliferative disease. Curr Hematol Rep 2003;2:202–8.
[45] Wimazal F, Krauth MT, Vales A, Bohm A, Agis H, Sonneck K, et al. Immunohistochemical detection of vascular endothelial growth factor (VEGF) in the bone marrow in patients with myelodysplastic syndromes: correlation between VEGF expression and the FAB category. Leuk Lymphoma 2006;47:451–60.
[46] Bertolini F, Mancuso P, Braidotti P, Shaked Y, Kerbel RS. The multiple personality disorder phenotype(s) of circulating endothelial cells in cancer. Biochim Biophys Acta 2009;1796:27–32.
[47] Mancuso P, Burlini A, Pruneri G, Goldhirsch A, Martinelli G, Bertolini F. Resting and activated endothelial cells are increased in the peripheral blood of cancer patients. Blood 2001;97:3658–61.
[48] Mancuso P, Antoniotti P, Quarna J, Calleri A, Rabascio C, Tacchetti C, et al. Validation of a standardized method for enumerating circulating endothelial cells and progenitors: flow cytometry and molecular and ultrastructural analyses. Clin Cancer Res 2009;15:267–73.
[49] Bertolini F, Marighetti P, Shaked Y. Cellular and soluble markers of tumor angiogenesis: from patient selection to the identification of the most appropriate postresistance therapy. Biochim Biophys Acta 2010;1806:131–7.
[50] Bertolini F, Paul S, Mancuso P, Monestiroli S, Gobbi A, Shaked Y, et al. Maximum tolerable dose and low-dose metronomic chemotherapy have opposite effects on the mobilization and viability of circulating endothelial progenitor cells. Cancer Res 2003;63:4342–6.
[51] Cortelezzi A, Fracchiolla NS, Mazzeo LM, Silvestris I, Pomati M, Somalvico F, et al. Endothelial precursors and mature endothelial cells are increased in the peripheral blood of myelodysplastic syndromes. Leuk Lymphoma 2005;46:1345–51.
[52] Yee KW, Hagey A, Verstovsek S, Cortes J, Garcia-Manero G, O'Brien SM, et al. Phase 1 study of ABT-751, a novel microtubule inhibitor, in patients with refractory hematologic malignancies. Clin Cancer Res 2005;11:6615–24.
[53] Della Porta MG, Malcovati L, Rigolin GM, Rosti V, Bonetti E, Travaglino E, et al. Immunophenotypic, cytogenetic and functional characterization of circulating endothelial cells in myelodysplastic syndromes. Leukemia 2008;22:530–7.
[54] Jain RK, Duda DG, Clark JW, Loeffler JS. Lessons from phase III clinical trials on anti-VEGF therapy for cancer. Nat Clin Pract Oncol 2006;3:24–40.
[55] Sekeres MA, List AF, Cuthbertson D, Paquette R, Ganetzky R, Latham D, et al. Phase I combination trial of lenalidomide and azacitidine in patients with higher-risk myelodysplastic syndromes. J Clin Oncol 2010;28:2253–8.