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SCIENCE DIRECT
Leukemia Research 30 (2006) 338–342
Leukemia Research
www.elsevier.com/locate/leukres

Brief communication

Treatment of myelodysplastic syndrome with a DNA methyltransferase inhibitor: Lack of evidence for induction of chromosomal instability

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Received 9 June 2005; received in revised form 29 July 2005; accepted 29 July 2005
Available online 12 September 2005

Abstract

In several large phase II trials, low-dose treatment with the azanucleoside 5-aza-2′-deoxycytidine (decitabine, DAC) resulted in complete hematologic and cytogenetic responses in 23 and 31% of MDS patients, respectively. The question of induction of chromosomal instability by this demethylating agent was addressed by serial karyotypic analyses. 53/122 DAC-treated patients had all normal metaphases at time of treatment start. In 46/53 patients, sequential cytogenetic analyses were performed. 9/46 patients (20%) acquired clonal chromosomal abnormalities during follow-up (4/9 transient). 8/9 abnormalities were gains or losses of entire chromosomes. The rate and pattern of cytogenetic evolution are thus not higher than in historical MDS cohorts not receiving specific treatment.

Keywords: DNA methylation; Myelodysplasia; Decitabine

1. Introduction

Cancer-related disturbances in regulated DNA methylation are an apparent conundrum: global hypomethylation (mostly of heterochromatic regions, but also of specific genes such as *H-RAS* and *C-MYC*) coexists with hypermethylation of a large and growing number of genes implicated in cancer [1]. Reversal of the silencing imposed by promoter methylation of these genes provided a strong rationale for developing pharmacological strategies to inhibit DNA methylation [2]. Two demethylating agents, 5-azacytidine (Vidaza) and 5-aza-2′-deoxycytidine (Decitabine, DAC) were developed employing low-dose schedules for the treatment of myelodysplasia (MDS), by virtue of their favorable non-hematologic toxicity profile and beneficial effects on hematopoiesis [3–5]. This approach was therefore particularly directed at elderly patients unable to tolerate aggressive treatment, such as standard induction chemotherapy or allogeneic blood stem cell transplantation. A randomized phase III study with subcutaneous 5-azacytidine has resulted in the first proof of change of the natural history of MDS with any treatment, and was associated with improvement in quality of life [6]. Recently, this drug was approved for the treatment of MDS. However, the development of demethylating agents also must take into account potential drawbacks of demethylation such as activation of oncogenes, induction of chromosomal instability and mutagenesis [1,7].

Different mouse models, developed to address the role of methylation changes in carcinogenesis and chromosomal instability, have yielded conflicting results: Hypomethylation in *APC*/*MIN* (adenomatosis polyposis coli/multiple intestinal neoplasia) mice by both haploinsufficiency of DNA methyltransferase 1 (*DNMT1*) and treatment with DAC results in protection from polyp formation in a dose-dependent manner [8]. In contrast, recent work by the same group employed a model with a hypomorphic *DNMT1* allele, resulting in

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0145-2126/$ – see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.leukres.2005.07.014

reduction of global methylation by 90%, and the development of malignant lymphoma with recurrent numerical chromosomal aberrations [9]. Despite the fact that in the rare ICF syndrome (for immunodeficiency, centromeric instability, facial anomalies) with global, lifelong DNA hypomethylation in humans [10], no increased rate of cancers has been noted, pharmacological demethylation over a prolonged period has been discussed as a potentially "double-edged sword" [11].

In three phase II trials of low-dose intravenous DAC in high-risk elderly MDS patients [4,5,12], we prospectively captured cytogenetic changes in order to: (i) define the rate of cytogenetic normalization, as a marker for treatment efficacy; (ii) determine the rate of cytogenetic evolution which is part of the natural history of MDS progression; (iii) capture any potential chromosomal abnormalities which might be associated with the treatment with this demethylating agent. While cytogenetic normalization was noted in 19/61 patients (31%) with an initially abnormal karyotype, patients relapsed with the identical clone in the large majority of these cases, whereas two patients showed clonal evolution at time of relapse and one patient relapsed with a different cytogenetic abnormality [17]. This data implied a rate of chromosomal evolution that is probably comparable to >140 MDS patients described in the literature which received supportive care, had normal karyotype and were followed with sequential cytogenetics [13–16,18–20].

2. Materials and methods

2.1. Patients

Between January 1991 and December 1999, a total of 122 patients with MDS were studied in three clinical protocols of low-dose Decitabine (provided by Pharmachemie BV, Haarlem, The Netherlands) approved by local Ethics Committees [4,5,12]. The first protocol (phase I/II study) used continuous infusion (c.i.) at two starting dose levels, subsequent protocols had a single dose level with infusions of 4 h duration three times daily. Starting doses were 50 mg/m^2/day c.i. for 3 days (total dose 150 mg/m^2, 15 patients) and 40 mg/m^2/day c.i. for 3 days (total dose 120 mg/m^2, 8 patients) in the phase I/II study. 15 mg/m^2 three times daily for 3 days (total dose 135 mg/m^2) were administered in both the phase II study (66 patients) and the subsequent "compassionate-use" protocol (35 patients). Inclusion criteria in all three protocols were: patients 18 years and older (no upper age limit) with MDS of subtype RAEB, RAEB-t, CMMoL, or transfusion-dependent RA and RARS. Thus younger patients in whom AML-type induction treatment was not feasible could also be treated. The compassionate-use program also allowed previous treatment with Decitabine. Courses were repeated every 6–8 weeks for up to eight courses. No dose reduction was allowed. Clinical results have been published [4,5,12].

2.2. Cytogenetic analyses

Bone marrow samples were obtained prior to treatment and per protocol at the end of every other course, at end of study, 6 and 12 months follow-up, and at suspected relapse. Karyotype analyses were performed on bone marrow cells from 122 patients after short-term cultivation (24–72 h). Whenever possible, at least 10–20 metaphases were analyzed. Chromosome aberrations were designated according to the recommendations of the International System for Human Cytogenetics Nomenclature. An abnormal clone was defined by the presence of at least two cells with the same structural rearrangement or extra chromosome, or at least three cells with the same missing chromosome.

3. Results and discussion

We evaluated the rate of cytogenetic evolution over time in MDS patients with a normal karyotype at time of DAC treatment start. 53/122 consecutively treated patients at three study centers (43%) had all normal metaphases at time of treatment start (Fig. 1). Patients received an average of 3.6 treatment courses (range, 1–15). In 46, at least one further cytogenetic analysis could be performed (average of 2.5 karyograms, range 1–9). 14/46 patients could be followed cytogenetically beyond 12 months from treatment start. 9/46 patients (20%) acquired clonal chromosomal abnormalities (five all abnormal, four mosaic) during follow-up (Table 1), 4/9 abnormalities were temporary and thus only normal metaphases detected during the last follow-up studies. 8/9 clonal abnormalities were gains or losses of entire chromosomes, with monosomy 7 (alone or in combination with other changes) observed in 3 patients, trisomy of chromosomes 11 and 21 in 2 patients each. In 3/9 patients, cytogenetic evolution occurred prior to 12 months from start of treatment (all developed mosaicism, which was temporary in two), while the other abnormalities (one mosaic, five with only abnormal metaphases) were detected at later timepoints. Median time to first abnormal karyotype was 14 months from treatment start (range 3–35, Fig. 1) (Tables 2 and 3).

Thus far, only a limited number of retrospective studies have addressed the rate and pattern of karyotypic evolution in MDS and no study has asked this question with regard to a specific treatment modality. Benitez et al. [16] described 33 patients, 18 of whom initially had normal karyotype. Of these, 4 (22%) developed chromosomal abnormalities (median time to first abnormality 12 months). Horiike et al. [14] followed 30 patients serially, of whom 3/15 (20%) with initially normal karyotype developed abnormalities (median time to first abnormality 15 months). Tricot et al. [13] noted chromosomal evolution in 5/30 patients with normal karyotype (17%), with a median time to first abnormality of 15 months. The rate of cytogenetic evolution noted in our present analysis is thus probably comparable with that of MDS cohorts not treated with a demethylating agent. In fact, considering