[haematologica reports]
2006;2(7):57-59

Azacytidine: first therapeutic opportunity for myelodysplastic syndromes

C. Finelli
C. Bosi
G. Martinelli
M. Baccarani

Istituto di Ematologia "L. & A. Seragnoli," Università di Bologna, Italy

Myelodysplastic syndromes (MDS) are clonal disorders characterized by ineffective hematopoiesis, wich can lead either to fatal cytopenia or acute myelogenous leukemia (AML). During the last 14 years, important progress has been made in the understanding of the biology and prognosis of MDS; risk-adapted treatment strategies were estabilished, according to the median age (60-75 years) of MDS patients and the individual history of the desease (number of cytopenias, cytogenetic changes, transfusion requirements).

The use of allogeneic bone marrow transplantation for MDS patients currently offers the only potentially curative treatment, but this kind of therapy is not available for the *typical* MDS patient, who is > 60 years of age. The development of new molecules directed against specific molecular targets, with few adverse effects, is the hope for the future.

DNA methylation of tumor suppressor genes is a frequent mechanism of transcriptional silencing in cancer.

5-Azacytidine, a ring analog of the naturally occurring pyrimidine nucleoside cytidine, showed some interesting and promising effects on cell differentiation, gene expression, and DNA synthesis and metabolism.

Since the early 1970s, Azacytidine at higher doses has been investigated for the treatment of acute leukemia.

Azacytidine is thought to have two main mechanisms of antineoplastic action: –a) cytotoxicity, resulting from incorporation into RNA and DNA; -b) hypomethylation, restoring normal growth control and differentiation in hematopoietic cells.

Induction of DNA hypomethylation appears to require lower Azacytidine doses than does cytotoxity, as the concentrations of Azacytidine required for maximum inhibition of DNA methylation *in vitro* do not suppress DNA synthesis.

Upon uptake by cells, Azacytidine is phosphorylated to 5-azacytidine-monophosphate by uridine-cytidine-kinase and then to diphosphate by pyrimidine-monophosphate and diphosphate-kinases, respectively.

5-Azacytidine-triphosphate is incorporated into RNA, disrupting nuclear and cytoplasmatic RNA metabolism and inhibiting protein synthesis.

5-Azacitidine-diphosphate is reduced by ribonucleotite-reductase to 5-aza-deoxycytidine diphosphate, wich is then phosphorilated by nucleoside-diphosphate-kinase to 5-azadeoxycitidine-triphosphate, which is incorporated into DNA; as a result, DNA sinthesis is inhibited.

Azacytidine is most toxic during the S-phase of cell cycle, but the predominant mechanism of cytotoxicity has not been estabilished. Azacytidine inhibits methylation of replicating DNA by stoichiometic binding with DNA-methyltransferase 1, resulting in DNA hypomethylation.

DNA hypermethylation at the CpG islands has been described in myelodysplastic syndromes, acute myelogenous leukemia, and other malignancies[1-4] (Figure 1 e 2).

The results of three clinical studies, conducted by the Cancer and Leukemia Group B (CALGB), demonstrated the effectiveness of Azacytidine in Myelodysplastic Syndromes, and led to the licensing of the drug in the US in 2004. Two studies were single-arm trials; the third study had a control arm.[5-7]

The controlled study was a randomized, open–label, phase III, multicenter trial, in which 99 patients were randomized to Azacytidine treatment, and 92 were randomized to best supportive care. Azacytidine was administered via subcutaneous injection, at starting dose of 75 mg/mq/day for 7 days in each 28-day cycle. An oral 5-HT antagonist (granisetron) was given as antiemetic prior to each injection. The Azacytidine dose was planned to be decreased either for hematological toxicities or decreased renal function. Study partecipants included patients with all five MDS subtypes, following the FAB classification. The patients initially enrolled in the control

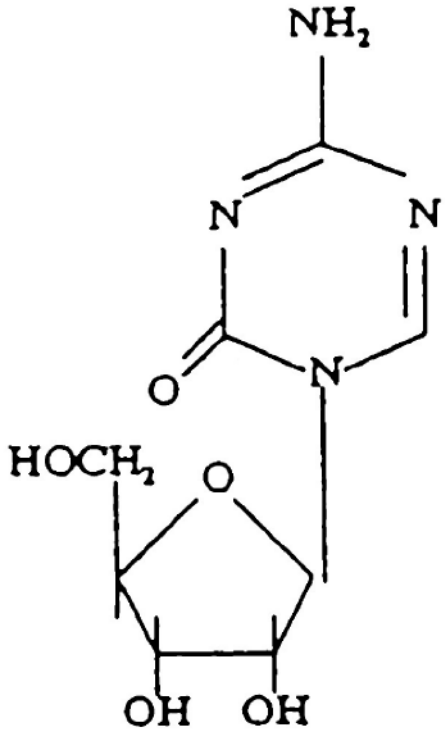

Figure 1. Molecular structure of Azacytidine.

arm were allowed to cross over to treatment with Azacytidine, if they met pre-specified criteria of disease progression (i.e.: worsening cytopenias and transfusion needs, major hemorrhages requiring platelets transfusions, clinical infections with neutropenia requiring antibiotics).

Haematologic responses occurred in 60% of the patients of the treatment arm (complete remission-CR: 7%; partial remission-PR: 16%; haematologic improvement: 37%) as compared to 5 % (haematologic improvement) of the patients of the control arm, only receiving supportive care.

The median time to leukemic transformation or death was of 21 months for the patients of the treatment arm, as compared to 13 months for the patients of the control arm. Transformation to acute myelogenous leukaemia (AML) occurred as the first event in 15 % of the patients on the Azacytidine arm, and in 38 % of the patients only receiving supportive care. Eliminating the confounding effect of early cross-over to Azacytidine, a landmark analysis after 6 months showed median survival of an additional 18 months for Azacytidine and 11 months for supportive care (p=0.03). The median duration of response, among the patients who achieved CR, PR, or haematologic improvement, was of 15 months. Quality-of-life assessment found significant major advantages in physical function, symptoms, and psychological state for patients initially randomized to Azacytidine.

The most evident benefit of haematologic response was observed in the patients who were transfusion–dependent at the start of treatment. The patients who were dependent on RBC and/or platelet transfusions at the study entry lost the need for transfusions for the duration of CR or PR. Among the patients taking Azacytidine, the mean number of RBC transfusions ini-

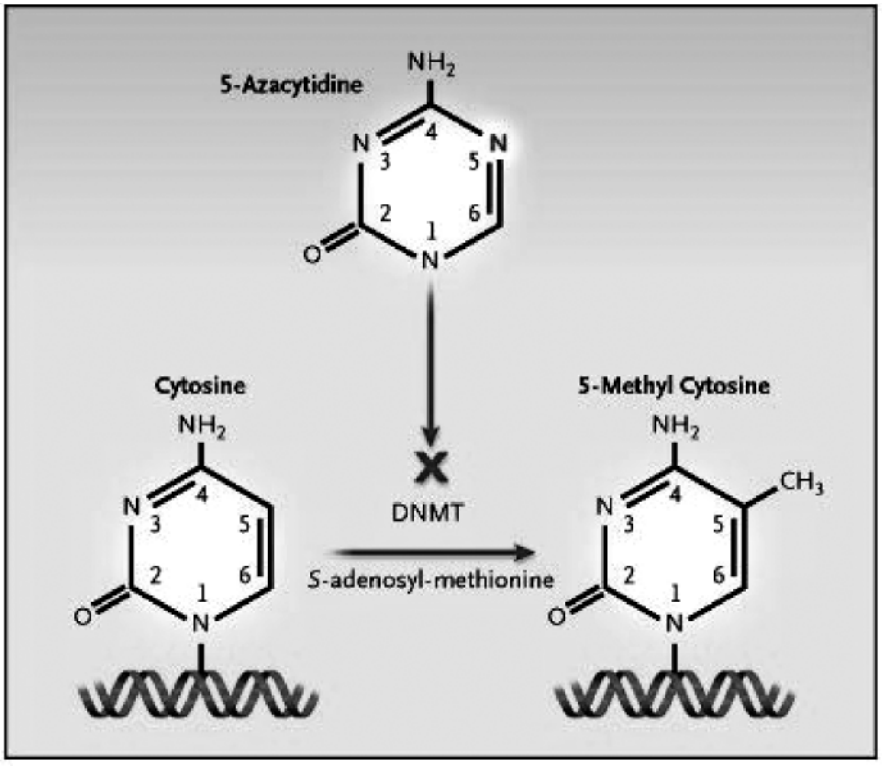

Figure 2.The mechanism of action of 5-Azacytidine.

tially increased, during the first month of treatment, but thereafter significantly declined, whereas the mean number of transfusions remained stable, or even increased, among the patients on supportive care.

The haematologic response became evident as from the third or fourth month of Azacytidine treatment; an increase in platelet count, hemoglobin, or WBC, and/or a decrease in bone marrow blast percentage, were observed by the fifth treatment cycle in more than 90% of the patients.

Serious adverse events (SAE) occurred in nearly 60% of the Azacytidine-treated patients and in 36% of the control- arm patients. In both arms, the most common SAE, resulting in hospitalization, were: thrombocytopenia, febrile neutropenia, fever, and pneumonia. No deaths were attributed to Azacytidine.

Gastrointestinal events (nausea, vomiting, diarrhea, constipation, and anorexia), hematologic events (neutropenia, fever, ecchymoses, and petechiae), injection-site events, arthralgia, cough, dyspnoea, headache, weakness, dizziness, and insomnia, were more commonly reported by the patients treated with Azacytidine as compared to the patients of the control arm. The greater proportion of adverse events occurred during the first two months of treatment; the incidence of adverse events subsequently decreased, as from the third cycle of therapy, owing to the administration of appropriate concomitant medications.[7-10]

In summary, Azacytidine is an active agent that provides a clinically relevant benefit (complete remission, partial remission or haematologic improvement) in nearly 60% of patients with MDS. Azacytidine treatment results in significantly higher responses rates, improved quality of life, reduced risk of leukemic transformation, and improved survival, as compared to supportive care alone. Therefore, Azacytidine provides a new treatment option, that is more effective than supportive care alone, for patients with all MDS subtypes.[10]

References

1. Christman J, Mendelsohn N, Herzog D, et al. Effect of 5-azacytidine on differentiation and Dna methylation in human promyelocitic leukemia cells. Cancer Res 1983;43:763-9.
2. Creusot F, Acs G, et al. Inhibition of Dna methyltransferase and induction of Friend erytroleukemia cell differentiation by 5-Aza-cytidine and 5-Aza-2-deoxycytidine J Biol Chem 1982;257:2041-8.
3. Fenaux P, et al. Inhibitors of Dna methylation :beyond myelodisplastic syndromes. Nat Clin Pract Oncol 2005 Dec;suppl 1:536-44.
4. Baylin SB, et al. Dna methylation and gene silencing in cancer. Nat Clin Pract Oncol 2005 Dec;suppl 1:4-11
5. Silverman et al. Effects of treatment with 5 –Azacytidine on the *in vivo* and *in vitro* hematopoiesis in patients with myelosplastic syndrome. Leukemia 1993;7:21-9.
6. Vonhoff D, Stark M, Muggia F. 5 Azacytidine, a new anticancer drug with effetiveness in acute myelogenous leukemia. Ann Intern Med 1976;85:237-45.
7. Glover AB, Leyland-Jones A, et al. 5 Azacytidine.10 years later. Cancer Treat Rep1987;71:737-46.
8. Jeffrey Gryn, Zella R, et al. Treatment of Myelodisplastic syndromes with 5-Azacytidine. Leukemia Research 2002;26:893-7.
9. Issa JP, et al. Optimizing Therapy with Methylation inhibitors in myelodiplastic syndromes: dose, duration,and patient selection. Nat Clin Pract Oncol 2005 Dec;suppl 1:24-9.
10. Silvermann, et al. Randomized ControlledTrial of Azacytidine in patients with the Myelodisplastic Syndrome: a study of the Cancer and Leukemia Group B. J Clin Oncol 2002;20:2429-40.