

Azacitidine: A novel drug for myelodysplastic syndrome

Myelodysplastic syndrome (MDS) is a group of diseases showing different outcomes depending on its clinical presentations, which varies from a mild to very aggressive form of malignancy. It is a group of clonal cell disorder characterized by maturation defects resulting in ineffective haemopoiesis and an increased risk of transformation to acute myeloblastic leukemia. Bone marrow is completely or partially filled by mutant pluripotent cells retaining the property of differentiation into blood cells. The resulted abnormal clone of cells in the marrow can undergo mutation leading to acute myeloid leukemia (AML). This is a disorder of advanced age usually presenting with the complaints of unexplained anemia, weakness, infections, acute reticulocytopenia, hemorrhages and pancytopenia. Progression to AML is seen in 10–40%. The median survival depends on the type of MDS karyotype and varies from 9 to 29 months. Repeated blood transfusions were the main treatment modality.

Many researchers reported that DNA hypermethylation at the *cpg* islands might be a key factor in diseases such as MDS, AML, and other malignancies. Drugs targeting DNA hypermethylation, can be promising and azacitidine, a DNA methyl transferase inhibitor has shown a good response in all types of MDS and also in transfusion-dependent varieties. Sixty-six percentage of patients can become transfusion dependent and patients with transfusion dependency lost the need for future transfusions.

Azacitidine with less and well controllable side effects can become the main stay of treatment in MDS. The present treatment of this disease such as bone marrow transplantation, GM-CSF therapy and interferons are costly for patients from both developing and developed countries.

Chemical structure

5-Azacitidine is an analogue of cytidine belonging to the pyrimidines. The position five of cytidine ring determines the functional importance of the compound. Changes in the functional groups at that position separates them into different compounds such as 5-azacytidine, 5-aza deoxycytidine, pseudosocytidine and 5-fluoro-2'-deoxycytidine. All these drugs are potential inhibitors of DNA hypermethylation.

The hypomethylating effects of these agents depend on altered c5 position, where as other cytidine analogues such as ara-C, 6-azacytidine and gemcitabine will not show this property.

A unique property of getting hydrolyzed in neutral and basic media was reported, which compels the preparation of fresh drug regularly during prolonged infusions.^[1]

Mechanism of action

Azacitidine identified as DNA and RNA methyl transferase inhibitor resulting in hypomethylation. Hypo or demethylation is a key mechanism exploited by malignant cells for silencing the antioncogenes without significantly affecting the codon sequence. This mechanism will silence the expression of

oncogenes such as p16, p15, E-cadherin, nMLH1, UHL, etc.^[2]. There are mainly two mechanisms of action.

Cytotoxicity is due to its incorporation into RNA and DNA. Hypomethylation of RNA and DNA is another mechanism. The cytotoxic effect needs higher amount of drug than hypomethylating effect^[3]. After the uptake from the cancer cells 5-azacytidine is converted into 5'-azacytidine monophosphate by receiving a phosphate group catalysed by the enzyme uridine-cytidine kinase and further converted to diphosphate by cytosine monophosphate kinase and to triphosphate with the help of enzyme diphosphate kinase.

The drug gets incorporated into the RNA and causes disassembly of polyribosomes, disrupts the methylation and acceptor function of tRNA. This in turn results in inhibition of protein production and interrupts the nucleic acid metabolism in cytoplasm and nucleus^[4,5]. Further, 5-azacytidine may undergo reduction by *ribonucleotide reductase* to form 5-aza-deoxycytidine diphosphate. This congener enters into DNA resulting in its synthesis inhibition. The demethylation or inhibition of methylation is critical in the pathogenesis many haematological malignancies. The drug covalently block DNA methyl transferase-1 (DNMT1) resulting in DNA hypomethylation. Drugs inhibiting histone de-acetylation could further decrease DNMT₁ activity when given along with these drugs.^[6]

Azacitidine acts mainly on rapidly dividing cells and most active in synthetic phase of cell cycle. Azacytidine as said above exerts its activity by re-expression of silenced genes. This can induce differentiation and activation of B- and T-lymphocytes^[7], and helps in differentiation of B-lymphocytes to immunoglobulin. Azacitidine also helps in induction of synthesis of IL-4 and IL-6.^[8]

Pharmacokinetics

Azacitidine is absorbed rapidly by the subcutaneous route. Maximum plasma concentration is reached after 30 min of subcutaneous administration and 11 min after a 10 min i.v. infusion. The mean concentration of subcutaneous route is only 25% that of i.v. administration and bioavailability is 89%. The plasma half-life varies with routes of administration. On i.v. infusion half-life is 22 min and on s.c. route 41 min. Volume of distribution is 76 liters which is more than the total body water and the drug is will be distributed in all tissues.

Azacitidine is metabolized by spontaneous hydrolysis and deamination by an enzyme *cytidine deaminase*. The metabolites and the drug itself are excreted mainly through kidney. The excretion again depends on the routes of administration, on i.v. -85% and after s.c. route-50% elimination seen. Less than 1% is excreted through feces. Mean elimination half-life is about 4 h with both types of administration. Azacitidine is neither a substrate, inhibitor nor an inducer of metabolizing enzymes. Literature is not available about the interactions with other drugs.^[9]

Indications

Azacitidine is the only drug approved for the treatment of MDS in May 2004 by U.S.F.D.A. as well given an orphan drug status.^[9] Azacitidine can be used for the treatment all FAB classified types of MDS such as:

1. Refractory anemia (RA) or refractory anemia with ringed sideroblasts (RARS). (if accompanied by neutropenia or thrombocytopenia or requiring transfusions).
2. Refractory anemia with excess blasts (RAEB).
3. Refractory anemia in transformation (RAEB-T).
4. Chronic myelomonocytic leukemia (CMML).

As MDS is usually seen in older individuals, other modes of treatment such as chemotherapy, stem cell transplantations said to be more aggressive modes, are not useful for most of the patients.

The combinations with topoisomerase inhibitors like topotecan may have synergistic activity. The combination may greatly reduce the number of cancer cell and clones, and inhibit the growth and synthesis to a higher extent. A greater response may be obtained only after 3.8 courses of treatment, which indicates the need for repeated courses for the success of the therapy. Azacitidine may also prevent the transformation of MDS to a full-blown AML. Interestingly in many clinical trials azacitidine-induced remission in several malignancies as many as 7 of 11 patients of breast carcinoma, two of five of melanoma and two of six of colon cancer.^[10]

Adverse drug reactions

The MDS itself can be an explanation for many adverse drug reactions reported with this drug. Hematological symptoms such as thrombocytopenia, febrile neutropenia, rigors, petechiae and myelosuppression. Most of the ADRs of azacitidine can be managed.

The most common side effects are gastrointestinal such as nausea, vomiting, diarrhea, constipation and anorexia. CNS side effects such as headache, weakness, fever, dizziness, and insomnia are also seen. Many injection site events were also noted. Pneumonia, cough and dyspnea are the respiratory adverse effects.

Serious adverse effects were found in more than 60% of azacitidine-treated patients than in controls (36%) in several studies. Most of the side effects were thrombocytopenia, febrile neutropenia pneumonia and fever. Majority were reported in first two cycles of therapy.

Most common causes of azacitidine discontinuation are hematological side effects such as neutropenia, leucopenia and thrombocytopenia.

Several liver function abnormalities can also be seen in patients with previously diagnosed liver cirrhosis and patients with inter current illnesses. Renal failure was noted in patients with hypotension and sepsis. Gender differences favourable to male with respect to ADRs are seen. In females, there can be more ADRs such as vomiting, diarrhea, hemolytic anemia, injection site erythema, arthralgia and postprocedural hemorrhage.^[11]

Clinical trials

A randomized controlled, open label, phase III, multicentre study administered a dose of azacitidine 75 mg/m²/day for 7

days in each 28-day cycle for 99 patients of all five types of MDS patients. Control arm was given supportive care. About 55% of patients did cross over to the observation arm during the study. Overall response rate was taken as the primary end point and was around 15.7%, it was 12.8% in the patients who did cross over.

There were also single arm trials. In one study, 72 patients with RAEB, RAEB-T and CMMoL were given the same drug dosage regimen as above. The response rate was 12.7% excluding the patients with the diagnosis of AML and overall response rate of 13.9%.

In other study, 48 patients were selected with the diagnosis of RAEB and RAEB-T and were given same dosage regimen, but were given intravenously instead of subcutaneous administration. The response rate excluding AML patients and overall response rates were 19.1% and 18.8%, respectively.^{[11]-[13]}

Conclusion

Azacitidine, a DNA methyl transferase inhibitor, is the first drug to be approved for the treatment of MDS by U.S.F.D.A. It causes adverse drug reactions that can be manageable. Dosage regimen, clinical trials and safety of the drug appear to be promising but it is premature to come to a conclusion before post marketing surveillance shows the real picture.

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