REVIEW

Decitabine: A Historical Review of the Development Process of an Epigenetic Drug

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ABSTRACT
Decitabine (5-aza-2p-deoxycytidine) is a hypomethylation agent with a double-action mechanism, these are the reactivation of silenced genes; exhibiting differentiation at low doses and showing cytotoxicity at high doses. Decitabine was used as a classic anticancer drug in the original studies in the 1980s, 1500 to 2500 mg/m2 per cycle was the maximum clinically tolerated dose. The dosage was reassessed after a better understanding of epigenetics in cancer and the role of decitabine in epigenetic (hypomethylation) therapy was obtained, in about 1/20th of the previous doses (i.e., ‘optimal biological’ doses modulating hypomethylation). It has been found that decitabine (100 to 150 mg / m2 per cycle) can be used in patients with myelodysplastic syndromes (MDS) and other myeloid tumors, with manageable side effects. Combination therapies which amplify the epigenetic effect of decitabine will most likely improve the patient responses and allow it to be used in the treatment of other malignancies.

KEYWORDS: Decitabine, Epigenetics, Oncology.

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HISTORY OF THE DRUG
The basis of tumor therapy is formed by surgery, radiotherapy and chemotherapy. Recently, cancer immunotherapy, which is considered to be one of the most promising and high potential cancer treatments in the 21st century, has gained worldwide. Modification of various immune system components, in addition to targeting checkpoint inhibitors such as CTLA-1 and PD-1, will also be possible.

DNA methylation is an epigenetic event that regulates chromatin compression and suppression of gene expression. Various genes are abnormally silenced by DNA methylation in cancer cells, including tumor suppressors, and control immune response and drug sensitivity. The inhibition of DNA methylation with cytidine analogs such as decitabine (5-aza-2p-deoxycytidine, Dacogen®, DAC for short) in turn reactivates the expression of genes which were silenced by hypermethylation. Decitabine (5-aza-2-deoxycytidine) is a unique cytosine analogue that can inhibit DNA methyltransferases, reverse methylation, and reactivate silenced genes. DAC participates in the copying of DNA and forms covalent bonds with the active sites of DNA methyltransferase irreversibly (1,2).

Decitabine was initially created in 1964, and in 1968 its potential antileukemic activity was reported (3). The interest of researchers in decitabine has increased with studies showing that decitabine is a stronger antileukemic agent in cytosine than arabinoside in mice, and clinical studies by Jones and Taylor reporting that murine embryonic cell line may induce terminal differentiation (4,5). Momparler et al. and Rivard et al. started the first clinical studies of decitabine in acute leukemia (6,7).

University of Texas M.D. Anderson Cancer Center leukemia group was the first to introduce decitabine to leukemia studies in the USA in 1992. In May 2006, Decitabine (Dacogen; SuperGen, Dublin, California) received approval from the U.S. Food and Drug Administration for the treatment of MDS and chronic myelomonocytic leukemia (CMML).
CLINICAL PHASE STUDIES

Early clinical studies
Twenty-one patients with advanced stage solid tumors were recruited to perform the Phase 1 pharmacokinetic study. The treatment, where the drug was given at dose ranges of 25 to 100 mg / m^2, and was infused for 3 hours every 8 hours, was repeated every 3 to 6 weeks. It was inferred that decitabine was largely and rapidly eliminated by metabolic processes from the fact that total urinary excretion was <1% of the administered dose (8).

Phase 1 studies
In the first clinical studies of decitabine in hematological malignancies, the dosage used was 1500 to 2500 mg / m^2 per course. In a phase 1 study which included thirty children with refractory or recurrent leukemia, where decitabine doses of 0.75 to 80 mg / kg were used, circulating blasts were reported to be significantly reduced. One complete remission was recorded in a patient with acute lymphocytic leukemia (ALL), where a dose of 37 mg / kg was given in a 36-hour infusion. In doses ranging from 31 to 81 mg / kg for 36 to 60 hours. Non-hematological toxicity was rare, 70% inhibition of DNA methylation detected (9,10).

Phase 2 studies
Two major phase 2 decitabine studies have been reported in myelodysplastic syndrome (MDS). In the European study conducted by Wijermans et al., it was administered to 169 elderly patients (mean total dose of 70 mg / m^2) with moderate-risk or high-risk MDS. The overall response rate was found to be 49% and the induction mortality rate was found to be 7%. In high-risk disease, the response rates were 51%, and it was 46% in intermediate disease. After two cycles, an improvement in thrombocytopenia was noted in 63% of patients. Various studies have been conducted to determine the tolerable dose and duration of administration in patients with solid tumors with different DAC based programs (1). Despite initial disappointing clinical results, interest in treating solid tumors with decitabine continued. Many preclinical studies have shown that the epithelial ovarian cancer development is associated with an abnormal accumulation of DNA methylation. In many phase I / II clinical studies, carboplatin and low-dose DAC were used in patients with platinum-resistant ovarian cancer. The Nephew group monitored DAC tolerability at an administration dose of 10 mg / m^2 per day continued for 5 days, which was followed by the administration of carboplatin on day 8 in patients with ovarian cancer. Using this regimen, 35% of 17 patients reported clinical responses and 53% of patients reported progression-free survival for 6 months (16,17). A clinical study has been conducted in patients with metastatic colorectal cancer. These patients were treated with DAC at 45 mg / m^2 on days 1 and 15 and panitumumab 6 mg / kg every 28 days on day 8 and 22. Excitingly, partial response developed in 10% of patients and stable disease was detected in 50% of patients. This outcome suggests that the combination of DAC with targeted therapy shows a clinical response and is well tolerated in metastatic colorectal patients (18). In the formation of lung cancer, epigenetic modifications have an important role. In some recent clinical studies, DAC was combined with chemotherapeutic drugs in patients with non-small cell lung cancer (NSCLC).

Phase 3 studies
The encouraging outcomes of the European phase 2 studies led to the conduction of a multi-institutional randomized phase 3 study in the United States. A hundred and seventy patients diagnosed with MDS were randomized to receive decitabine treatment, at a dose of 15 mg / m^2, which will be administered intravenously every 3 hours for 3 hours. Treatment days repeated every 6 weeks (at a dose of 135 mg / m^2 per cycle) or the best supportive care demonstrated a higher objective response rate to decitabine. Compared to supportive care response rate (17% versus 0% (P <.001)), all patients who responded to decitabine treatment had higher quality of life scores, and in the absence of growth factors, no red blood cell and platelet transfusion was required, an expected toxicity profile for this class of agents was well tolerated. The most common side effects were associated with current and severe myelosuppression.
Patients were treated with increasing doses of DAC (5–15 mg/m²) for a duration of ten days, combined with valproic acid (VPA) (10–20 mg/kg/day) on days 5 through 21 of a 28-day cycle. However, the clinical response was disappointing, because the neurological toxicity caused by VPA was unacceptable. There was only one patient who had stable disease (19).

**DRUG SIDE EFFECTS**

Most of the side effects associated with the use of decitabine have been hematological. However, myelosuppression, nausea, headache, vertigo, emesis, anxiety, cerebral hemorrhage, dyspnea, and systemic infections can be seen in decitabine use, similar to other chemotherapeutic agents. Still, as this drug is used more and more frequently, there is a potential theoretical concern about three different topics. DNA methylation plays an important role for normal development, and the gene expression changes caused by the use of this drug may lead to significant teratogenicity, clinical studies should consider this. Second, epigenetic silencing is a regular part of the normal cellular function of adult cells. Decitabine therapy can therefore potentially alter normal cellular physiology and have unusual short- or long-term side effects. Finally, data on hypomethylation of selected gene regions in cancer raises stimulating problems with the use of hypomethylation agents. In mouse models where excessive hypomethylation could be achieved, lymphomas appeared more frequently than expected. Decitabine can lead to in vitro chromosomal changes in the treated cells, and deep hypomethylation has been shown to be associated with characteristic karyotypic changes. The long-term effects of decitabine treatment need to be clearly monitored (20).

There is no official clinical drug interaction study with decitabine. There is a possibility of drug-drug interaction with other substances metabolized by sequential phosphorylation (via intracellular phosphokinase activity) and/or enzymes (such as cytidine deaminase) involved in inactivation of decitabine. Therefore, caution should be exercised when combining these active ingredients with decitabine. Since decitabine binds to plasma proteins to a negligible degree (<1%) in vitro, decitabine cannot be separated from plasma proteins by co-administered drugs. Decitabine is a weak inhibitor of P-glycoprotein (P-gp) mediated in vitro transport event and therefore, co-administered drugs are not expected to affect P-gp mediated transport.

**INHIBITION MECHANISM**

The antineoplastic effect of decitabine is due to its inclusion in the newly synthesized DNA. It has a dose-dependent dual mechanism of action and is an S-phase specific agent. Cytotoxic activity when administered at high doses is because of the covalent capture of the DNA methyltransferase enzyme into DNA. When given at lower doses, the antitumor effect is probably based on the ability of decitabine of inhibiting DNA hypermethylation and reactivating tumor suppressor genes. When administered at low dose, decitabine does not prevent the cell cycle progression of G1 phase cells to the S-phase. A low dose of decitabine, which is incorporated into DNA, leads to the capture and subsequent depletion of the DNA methyltransferase enzyme. Decitabine-induced hypomethylation resulted in re-expression of tumor suppressor genes, suppression of tumor growth and stimulation of cellular differentiation (4,12).

Decitabine is phosphorylated and included in DNA. Then, DNA binds to methyltransferases covalently, and traps the enzyme to the DNA, effectively acting as an irreversible inhibitor of the enzymatic activity. As a result, decitabine leads to a significant in vitro DNA hypomethylation, and in vivo and restores silenced gene expression. The detailed molecular mechanism of its action is still being researched. It is histone methylation that modifies the histones between DNA methylation, and each of these biochemical changes in a specific gene triggers the other, which in turn creates a self-reinforcing silencing cycle. This silencing cycle is cut with decitabine. Decitabine acts by reversing the silenced histone code in the tumor suppressor gene locus by causing hypomethylation. This dual effect (hypomethylation/histone changes) that decitabine exhibits may be the cause of its superior effect on gene expression compared to histone deacetylase inhibitors. Decitabine also demonstrates important effects on the expression of genes that are not silenced by the methylation of CpG islands. Decitabine induces p21 expression, which is a gene which does not show DNA hypermethylation in cancer. Decitabine has various other effects on the histone code, in addition to genes that show silencing by promoter-associated methylation. In bladder cancer cells, the significant and rapid remodeling of the heterochromatic areas of the decitabine-dependent p14ARF/p16INK4a locus, decreasing demethylated H3-K9 levels and increasing demethylated H3-K4 levels are observed. It also increases acetylation and H3-K4 methylation in the unmethylated p14 promoter, which suggests that it can induce chromatin remodeling independent of its effects on cytosine methylation. Other changes may be reactive, which occur due to the stress of exposure of the cells to a cytotoxic agent. Therefore, the final antineoplastic mechanism of decitabine treatment can be highly pleiotropic (21,22).

**STUDIES ON ITS USE WITH INNOVATIVE METHODS**

Decitabine can be combined with other agents to enhance its epigenetic effect. Combining decitabine with histone deacetylase inhibitors shows a synergistic effect in reactivating gene expression. Based on the demonstrated in vitro synergic activity, there are ongoing studies which are combining decitabine with vorinostat, valproic acid (VPA), depsipeptide and histone deacetylase inhibitors (12). In vivo, it was shown that decitabine can sensitize cells to the effects of biological therapy, for example retinoic acid, and can increase the expression of proapoptotic molecules (23,24).

Studies on the oral use of the drug are ongoing. Cedazuridine is a stable CDA inhibitor, which is stable in
pharmaceutical preparations and is active at low doses. In a study of myelodysplastic syndrome, oral decitabine in addition to cedazuridine mimicked the pharmacokinetics of intravenous decitabine, and showed a similar safety profile and dose-related demethylation. When compared to intravenous decitabine therapy for 5 days, the clinical responses were similar. It has been suggested that it can be used as an alternative to parenteral treatment or as a combination treatment with other new oral agents for various myeloid disorders (25). Venetoclax is a selective, potent, and orally bioavailable small molecule inhibitor of BCL-2. Venetoclax plus decitabine therapy appears to be a new, well-tolerated regimen with promising activity in the treatment of AML (26).

AUTHORS’ CONTRIBUTIONS

The participation of each author corresponds to the criteria of authorship and contributorship emphasized in the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly work in Medical Journals of the International Committee of Medical Journal Editors. Indeed, all the authors have actively participated in the redaction, the revision of the manuscript and provided approval for this final revised version.

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STATEMENT OF ETHICS

The authors have no ethical conflicts to disclose.

DISCLOSURE STATEMENT

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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