Journal of Current Pharma Research
(An Official Publication of Human Journals)
An International Peer Reviewed Journal For Pharmacy, Medical & Biological Science
DOI: 10.25166 CODEN: JCPRD6 NLM ID: 101744065
ISSN: Print: 2230-7834 Online: 2230-7842
SJIF Impact Factor: 6.913

Human Journals
Research Article
December 2022 Vol.:16, Issue:3

Preformulation Study of Decitabine

Journal of Current Pharma Research
(An Official Publication of Human Journals)
An International Peer Reviewed Journal For Pharmacy, Medical & Biological Science
DOI: 10.25166 CODEN: JCPRD6 NLM ID: 101744065

G Bala Kishore*[1], Suresh Kumar G V[2]

[1]Research Scholar Sunrise University, Alwar, Rajasthan, India

[2]Research Guide Sunrise University, Alwar, Rajasthan, India

Submitted: 01 December 2022
Accepted: 21 December 2022
Published: 25 December 2022

Keywords: Preformulation, Decitabine, anti-cancer

ABSTRACT

During preformulation study, it was confirmed that the active substance is Decitabine. Based on the study outcome, it was concluded that the possibility of developing aqueous formulations is not possible due to the severe degradation of hydrolytic impurity in the aqueous environment. Based on the unsatisfactory results of aqueous formulation attempts, nonaqueous formulation trials were attempted. The attempt of non-aqueous was able to give better results compared to aqueous formulations of Decitabine. However, still there is a scope to work to fine tune non-aqueous formulations of Decitabine concerning further control of impurities.

HUMAN JOURNALS
www.jcpr.humanjournals.com

INTRODUCTION:

The introduction part gave over the details required on the cancer and type of cancer[1-5]. Also the required important information related to the proposed research drug substance of Decitabine concerning its physical, chemical and biological properties was captured. The proposed research work being injectable dosage of anti-cancer drug, hence brief details on the parenteral dosage form, was also covered. The literature review was able to picturize the formulations of Decitabine which are published in the various patents and patent applications. Also the literature review revealed the reconstitution details on the various excipients being used in the proposed research work. Based on the literature survey carried, it is understood that there the present lyophilized[6-12] drug product has very shorter period after dilution with compatible fluids. Hence, there is a lacuna for the simple liquid and stable dosage form of Decitabine. It is concluded that there is a need to develop a ready-to-dilute and a simple stable dosage form of anti-cancer drug called Decitabine.

Need of the Study:

From the literature review, it was concluded that there is a requirement of carrying research work to present a simple and convenient solution and stable dosage form of Decitabine. It is understood that the available marketed product is a lyophilized one having very less shorter time for the infusion since the hydrolysis is causing a drug product to undergo severe degradation. Further, it would be difficult for a health care practitioner especially in the emergency cases when the primary reconstitution needs to be carried since there is a chance of dilution error. Since the drug substance is highly sensitive to temperature and further degradation is pH dependent and also higher degradation is seen in an aqueous environment and hence it was challenging to optimize a suitable solvent and it's quantity to be present in the formulation to make a stable liquid dosage form. Ready to dilute solution would aid in ease of use and avoids one step of reconstitution. Also, Ready to dilute solution would minimize/reduce the dosing errors resulting from reconstitution. As the Lyophilized process or technique is time-consuming process and also a tedious and requires considerable amount of energy during the process. Further, the lyophilization process is a cumbersome procedure which requires expensive technology. The cycle development, optimization and further scaling up to commercial level requires cost involvement. Hence, there was a need to develop a simple ready to dilute liquid formulation of Decitabine.

METHODOLOGY:

The methodology aspects covered the developmental aspects of aqueous and non-aqueous formulations. Further, the objective of methodology was to arrive an optimized composition concerning quality and quantity of solvents/vehicles to present a stable formulation. The methodology aspects also covered the pre-formulation aspects which confirmed that the tested active pharmaceutical ingredient is Decitabine. The various aspects of methodology is covered below.

Before initiating the formulation developmental activity, it is an important step to ensure that the physical and chemical properties of the drug molecule and other derived properties of the drug. Ensuring the compliance of an early-stage developmental activity plays an important role for the successful formulation work. This information would help to dictate many of the subsequent events and possible approaches in the development of the formulation.

Description

Physical appearance of Decitabine was evaluated and reported under results section.

Identification by IR spectroscopy

Fourier Transform Infra-Red Spectrum (FTIR)

The infrared spectra of decitabine were studied using a Fourier Transform Infrared (FTIR-ATR-Perkin Elmer 2000) spectrophotometer. IR Spectra was recorded using a frequency range of 400–4000 cm^{-1}, and averaged over 4 runs. A powdered sample was placed on the Attenuated Total Reflectance (ATR) crystal, and then compressed using an axial screw.

Solubility determination

A small quantity of Decitabine was dissolved in each of the required solvents.

Water, Methanol, Ethanol, and Dimethylsulfoxide [DMSO].

Table No. 1: Showing the USP definition of solubility

USP standards	
Soluble	10 to 30 parts of solvent
Sparingly soluble	30 to 100 parts solvent
Slightly soluble	100 to 1000 parts of
Insoluble	10,000 parts of solvents

Melting Point:

The melting point of a drug substance was measured using a 10° C./minute heating rate and a starting temperature of 40° C. in a DSC Q1000 model instrument. Thus, the DSC event resulted from the decomposition of decitabine.

XRPD: XRPD analysis of Decitabine has been studied for the drug substance.

Water content:

Karl Fischer titration is a classic titration method in analytical chemistry that uses coulometric or volumetric titration to determine trace amounts of water in a sample.

Karl Fischer titration was used as an analytical method for quantifying water content in the API and in drug and methanol was used as a solvent.

$$\text{Moisture content} = \frac{\text{Volume consumed x KF factor}}{\text{Weight taken}}$$

The water content was checked by auto Karl Fischer Titration and the results were reported.

pH (0.5% solution in water): pH of the 1% solution in water was determined using pH meter and the result is reported.

Hygroscopicity: The drug substance is hygroscopic in nature and environmental conditions such as temperature and humidity to induce polymorphic conversions. A hygroscopic study was performed to ascertain the relative stability of polymorphs concerning standard from by applying the relative stability of accelerated environmental conditions (i.e 75% RH at 40°C). Samples were accurately weighed and stored at 75% relative humidity (RH) at 40°C. Weight of samples was measured after 7, 14 and 28 days.

Analytical Method Development for Assay and Related Substances Test parameters.

ASSAY:

Principle: Reverse phase liquid chromatography with gradient elution and UV detector.

Table No.:2. Chromatographic Conditions

Column	**:** Inertsil ODS 3V, 250mm x 4.6 mm , 5µm.
Wavelength	**:** 240 nm
Flow rate	**:** 1.0 mL/min
Injection volume	**:** 5.0 µL
Run time	**:** 27.0 minutes
Column Oven Temperature	**:** 35°C
Auto Sample Temperature	**:** Ambient
Elution Mode	**:** Gradient

Table No.:3. Details of Gradient Programme

Time (Minutes)	**Mobile Phase-A(%)**	**Mobile Phase-B** (%)
0	100	0
3	100	0
10	85	15
18	50	50
18.01	100	0
27	100	0

Preparation of Mobile Phase-A:

Dissolve 1.54 g of ammonium acetate into 1940 mL of water. Add 60 mL of methanol and sonicateto degas.

Preparation of Mobile Phase-B:

Dissolve 0.77 g of Ammonium acetate in 400 mL of water. Add 600 mL of methanol and sonicate to degas.

Blank/Diluent: Dimethylsulfoxide (DMSO)

Preparation of Standard:

Weigh and transfer about 9.0 mg of Decitabine standard into I 00 mL volumetric flask. Dissolve and dilute to volume with diluent and mix well.

Preparation of Sample solution:

Reconstitute three vials of sample each with IO mL of diluent and transfer the whole content to I 00 mL volumetric flask. Rinse the sample vials for three times with diluent and then transfer the rinsed solution in to same volumetric flask. Dilute to volume with diluent and mix well. Allow the solution to settle for 10 minutes (to make the sol id particles settle down) and from the upper clear supernatant, pipette out 3.0 mL into 50 mL volumetric flask and dilute to volume with diluent and mix well.

Procedure:

Inject Blank (Diluent) (one injection) and Standard solution (five injections). Check the system suitability parameters.

System Suitability:

- The theoretical plates for the Decitabine peak from the first injection of Standard solution should be NLT 2000.
- The Tailing factor for the Decitabine peak from the first injection of Standard solution should beNMT2.0.
- The % RSD of the area response for Decitabine peak from five replicate injections of Standard solution should be NMT 2.0.

If System suitability criteria passes, then inject sample solution (two injections) and standard solution (one injection) as Bracketing standard for every six injections of the

Sample solution and at end of the sequence into the chromatograph and record the chromatograms.

Table No.: 4. Details of Injection Profile

S.No.	Solution Name	Number of injections
1	Blank (Diluent)	1
2	Standard solution	5
3	Sample Solution	2
4	Standard solution (BKT)	1

FORMULAE FOR CALCULATION:

a) Correlation Coefficient (r):

$$r = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{n(\sum x^2) - (\sum x)^2}\sqrt{n(\sum y^2) - (\sum y)^2}}$$

I= Sum of, x =Cone. Of the Componen t, y =Av. Area response ratio of component, n = number of observations

1. **Slope(a)=** **(nIXY-IXIY)** / **(n IX2-(I X)2)**

2. **The equation of straight line:** Y=aX+b

3. **Tailing factor (T): T= (a+b)/2a**

4. **Theoretical plates (N): N=16(VJWb)** ^{2}

5. **Intercept on the Y axis (b) =*Y- a X*, (*X* =mean values of X), (*Y* =mean values of Y)**

6. **Calculate the percent Assay of Decitabine by the following formula:**

Calculate Assay of Decitabine by using the following formula:

$$\%Assay = \frac{AT}{AS} \times \frac{ws}{DS} \times \frac{DT}{N} \times \frac{p}{100} \times \frac{100}{LC}$$

Where,

AT: Average area of Decitabine peak obtained from the Sample solution chromatograms

AS: Average area of Decitabine peak from five replicate injections of Standard solution chromatograms

WS: Weight of Decitabine Standard taken (in mg)

OS: Dilution of Standard solution (in mL)

OT: Dilution of Sample solution (in mL)

N: Number of vials of Decitabine Injection

P: Potency of Decitabine standard (¾ w/w, on as is basis)

LC: The labelled amount of Decitabine (50 mg/vial)

Sample Chromatograms of Assay Method Development of Decitabine Injection

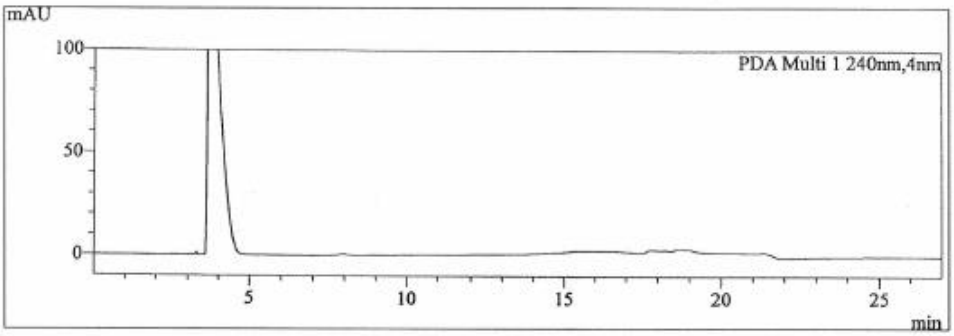

Figure No.:1. Chromatogram of Blank

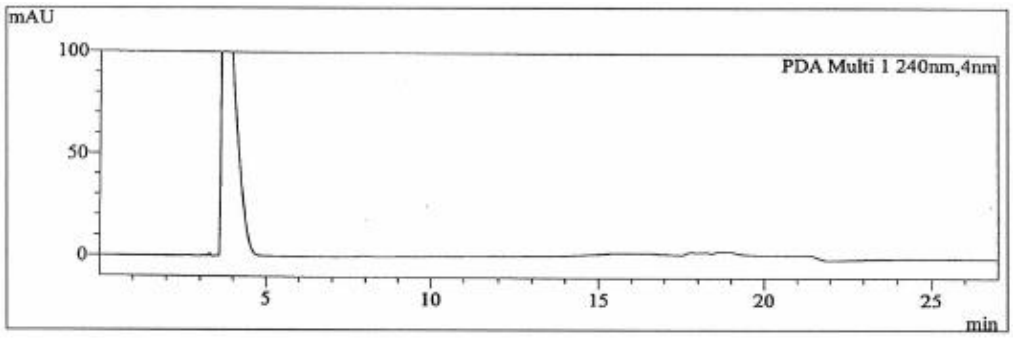

Figure No.:2. Chromatogram of Placebo

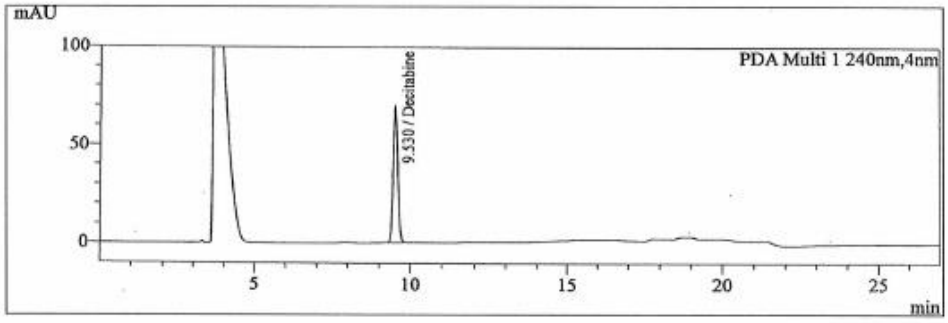

Figure No.:3. Chromatogram of Standard Solution

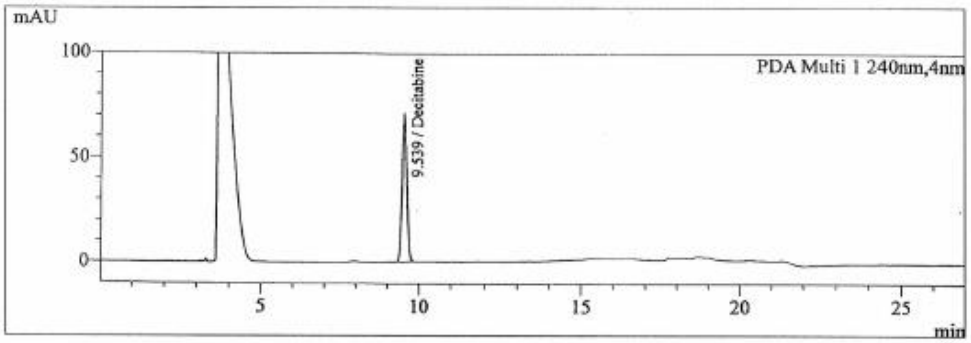

Figure No.:4. Chromatogram of Sample Solution

RESULTS:

Table No.:5. PREFORMULATION STUDY RESULS

<table>
<tr><th>Sl. No.</th><th>Test parameter</th><th>Results</th></tr>
<tr><td>1</td><td>Description</td><td>White fine powder</td></tr>
<tr><td>2</td><td>Solubility</td><td>
<table>
<tr><th>Buffer pH</th><th>Quantity Dissolved at 25°C (mg/mL)</th></tr>
<tr><td>1.2</td><td>0.1g/10 mL</td></tr>
<tr><td>2.0</td><td>0.1g/10 mL</td></tr>
<tr><td>4.0</td><td>0.1g/10 mL</td></tr>
<tr><td>6.0</td><td>0.1g/10 mL</td></tr>
<tr><td>8.0</td><td>0.1g/10 mL</td></tr>
</table>
</td></tr>
<tr><td>3</td><td>Identification by IR</td><td>The sample spectrum exhibited maxima only at the same wave length as that of standard spectrum. The IR spectrum of Decitabine</td></tr>
<tr><td>4</td><td>Melting Point</td><td>Between 195°C and 205°C</td></tr>
<tr><td>5</td><td>Hygroscopic study</td><td>The material found hygroscopic.</td></tr>
<tr><td>6</td><td>XRD study</td><td>The result conformed material is amorphous</td></tr>
<tr><td>7</td><td>Water content</td><td>Not More Than 1.0%</td></tr>
<tr><td>8</td><td>pH</td><td>Between 6.0 and 7.0</td></tr>
</table>

Solubility: Found Soluble in DMSO at 90 mg/mL; soluble in ethanol at 2 mg/mL with warming; soluble in water at 25 mg/mL with warming; buffers, serum, or other additives may increase or decrease the aqueous.

Identification by IR

The decitabine drug sample exhibited characteristic peaks such as C–H stretching (alkane) at 2918 cm^{-1} and stretching of the NH group (3467 cm^{-1}).

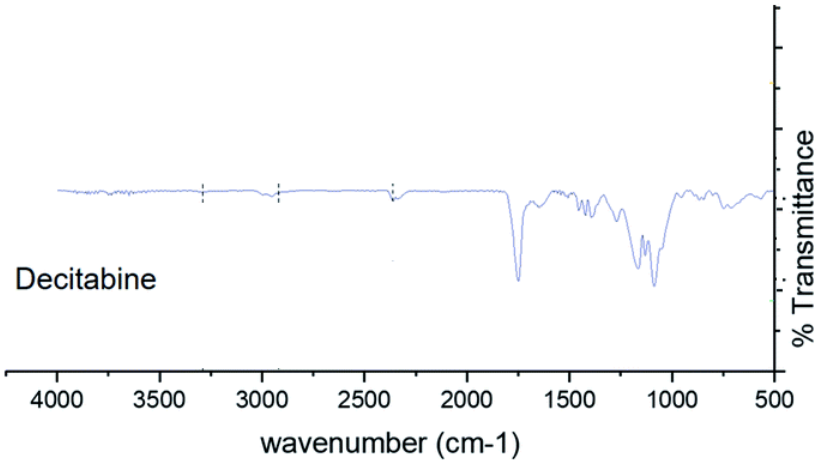

Figure No.: 5. IR Spectrum of Decitabine

Melting point by DSC chromatogram

By the onset of DSC graph of Decitabine where it was observed that the melting onset falls at about 200°C and a following exothermic decomposition of the sample.

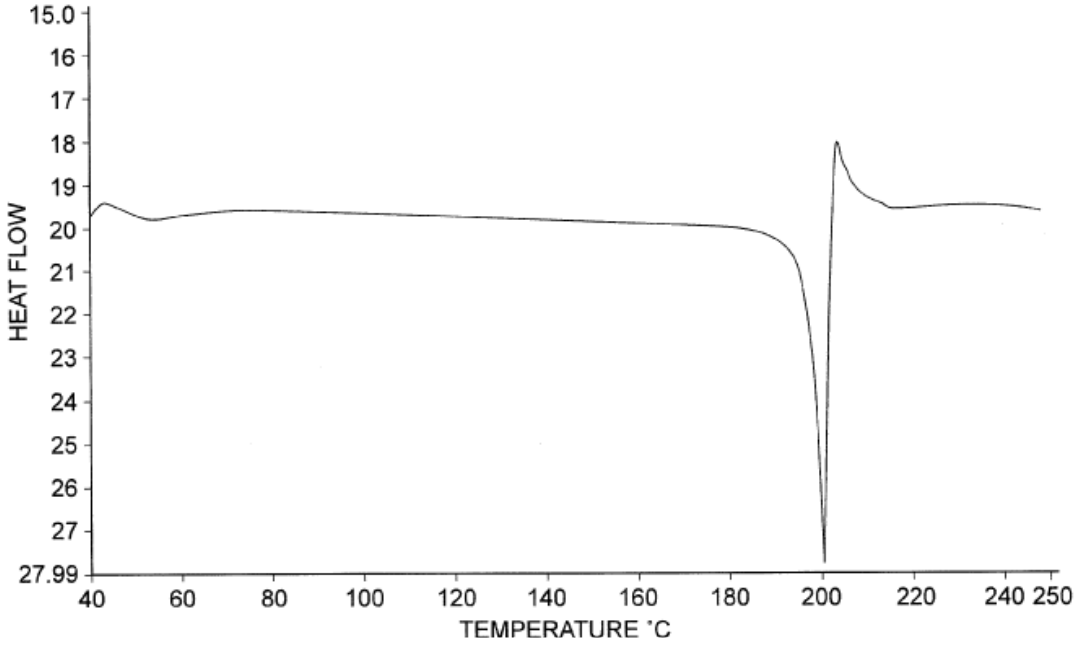

Figure No.:6. DSC Theromgram of Decitabine

Table No.:6. Hygroscopic Study Results**.**

API	Weight (mg)				Weight Change (%)			
	0 Day	7^{th} Day	21^{st} Day	28^{th} Day	0 Day	7^{th} Day	21^{st} Day	28^{th} Day
Decitabine	200.00	206.8	218.9	234.5	Nil	3.4%	9.45	17.25

The drug substance is hygroscopic nature in high humidity conditions. The drug substance has absorbed the moisture around 17.00% of its weight after 28 Days. Hence the substance is found highly hygroscopic and unstable at high humidity condition.

XRD Chromatogram

The diffraction peak visible to about 37.62° is due to the sample container. crystalline decitabine having characteristic XRPD peaks located at approximately 7.0, 13.0, 14.3, 18.5, 21.5 and 24.5±0.2° 2θ, or having peaks located substantially as shown in the XRD diffractogram.

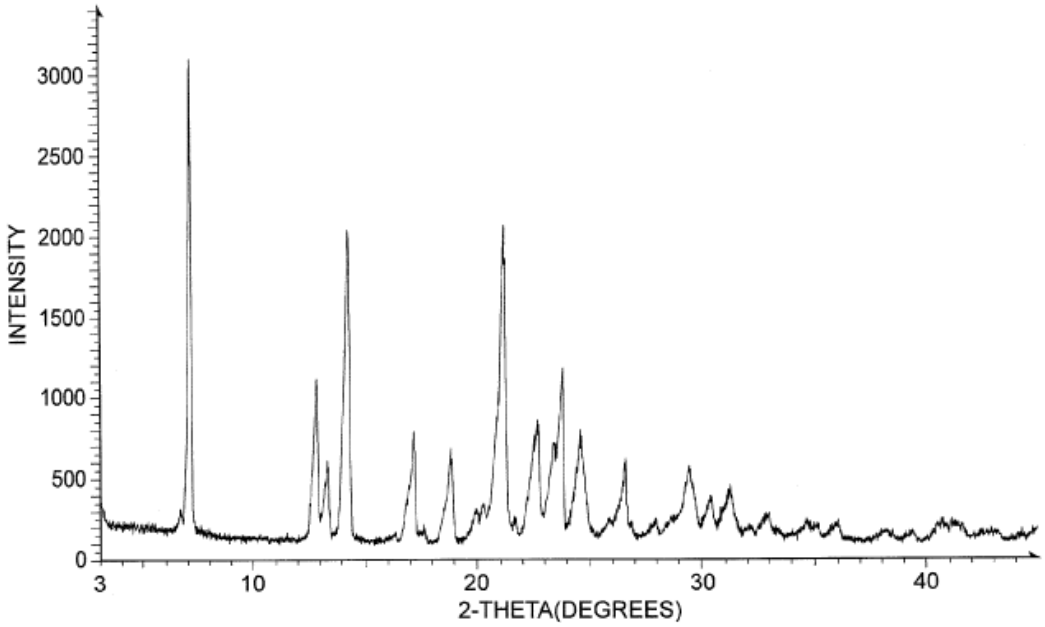

Fig No.: 7. XRD diffractogram of Decitabine

Water Content: The water content of the drug substance Decitabine was measured by using KF titrator and result was 0.51%.

pH: pH of the drug substance was measured using 0.5% of the concertation in carbon dioxide free water and the result is 6.42.

CONCLUSION:

During preformulation study, it was confirmed that the active substance is Decitabine. Based on the study outcome, It was concluded that the possibility of developing aqueous formulations is not possible due to severe degradation of hydrolytic impurity in the aqueous environment. Based on the un satisfactory results of aqueous formulation attempts, non aqueous formulations trials were attempted. The attempt of non-aqueous was able to give better results compared to aqueous formulations of Decitabine. However, still there is a scope to work to fine tune non-aqueous formulations of Decitabine concerning further control of impurities.

REFERENCES:

1. Decitabine. National Center for Biotechnology Information. Retrieved September 24, 2016.
2.Jump up to: "EC Approves Marketing Authorization Of DACOGEN For Acute Myeloid Leukemia". 2012-09-28. Retrieved 28 September 2012.
3.Ravandi, F.; Kantarjian, J. E.; Issa, S.; Jabbour, S.; Santos, G.; McCue, D.; Garcia-Manero, F. P. S.; Pierce, E.; O'Brien, J. P.; Cortés, J. E.; Ravandi, F. (2010). "Feasibility of Therapy with Hypomethylating Agents in Patients with Renal Insufficiency". Clinical Lymphoma, Myeloma & Leukemia. 10 (3): 205–210. doi:10.3816/CLML.2010.n.032. PMC 3726276. PMID20511166.
4. Kantarjian H, Issa JP, Rosenfeld CS, et al. (April 2006). "Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study". Cancer. 106 (8): 1794–1803. doi:10.1002/cncr.21792. PMID 16532500.
5. Kantarjian HM, O'Brien S, Cortes J, et al. (August 2003). "Results of decitabine (5-aza-2'deoxycytidine) therapy in 130 patients with chronic myelogenous leukemia". Cancer. 98 (3): 522–528. doi:10.1002/cncr.11543. PMID 12879469.
6. Dacogen Assessment report. European Medicines Agency, 19 July 2012 EMA/620205/2012.
7. Decitabine. National Center for Biotechnology Information. Retrieved September 24, 2016.
8. Jump up to: "EC Approves Marketing Authorization of DACOGEN For Acute Myeloid Leukemia". 2012-09-28. Retrieved 28 September 2012.
9. Ravandi, F.; Kantarjian, J. E.; Issa, S.; Jabbour, S.; Santos, G.; McCue, D.; Garcia-Manero, F. P. S.; Pierce, E.; O'Brien, J. P.; Cortés, J. E.; Ravandi, F. (2010). "Feasibility of Therapy with Hypomethylating Agents in Patients with Renal Insufficiency". Clinical Lymphoma, Myeloma & Leukemia. 10 (3): 205–210. doi:10.3816/CLML.2010.n.032. PMC 3726276. PMID 20511166.
10. Kantarjian H, Issa JP, Rosenfeld CS, et al. (April 2006). "Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study". Cancer. 106 (8): 1794–1803. doi:10.1002/cncr.21792. PMID 16532500.
11. Kantarjian HM, O'Brien S, Cortes J, et al. (August 2003). "Results of decitabine (5-aza-2'deoxycytidine) therapy in 130 patients with chronic myelogenous leukemia". Cancer. 98 (3): 522–528. doi:10.1002/cncr.11543. PMID 12879469.
12: Dacogen Prescribing Information issued by the USFDA.