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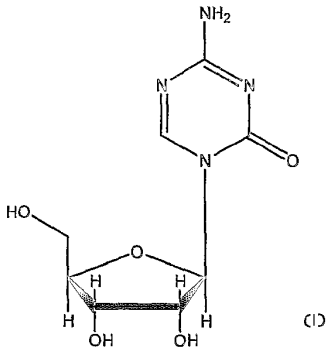
(54) Title: METHODS FOR ISOLATING CRYSTALLINE FORM I OF 5-AZACYTIDINE




(I)

(57) Abstract: The invention includes methods for isolating crystalline Form I of 5-azacytidine substantially free of other forms, wherein 5-azacytidine is represented by the formula: The invention also includes pharmaceutical compositions comprising Form I of 5-azacytidine.

# METHODS FOR ISOLATING CRYSTALLINE FORM I OF 5-AZACYTIDINE

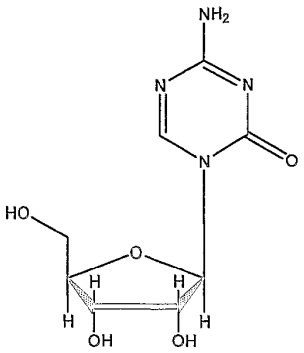
## Field of the Invention

The invention relates to the isolation of crystalline polymorphic Form I of 5-azacytidine (also known as azacitidine and 4-amino-1-β-D-ribofuranosyl-S-triazin-2(1*H*)-one). 5-azacytidine may be used in the treatment of disease, including the treatment of myelodysplastic syndromes (MDS).

## Background of the Invention

Polymorphs exist as two or more crystalline phases that have different arrangements and/or different conformations of the molecule in a crystal lattice. When a solvent molecule(s) is contained within the crystal lattice the resulting crystal is called a pseudopolymorph, or solvate. If the solvent molecule(s) within the crystal structure is a water molecule, then the pseudopolymorph/solvate is called a hydrate. The polymorphic and pseudopolymorphic solids display different physical properties, including those due to packing, and various thermodynamic, spectroscopic, interfacial and mechanical properties (See H. Brittain, Polymorphism in Pharmaceutical Solids, Marcel Dekker, New York, NY, 1999, pp. 1-2). Polymorphic and pseudopolymorphic forms of the drug substance (also known as the "active pharmaceutical ingredient" (API)), as administered by itself or formulated as a drug product (also known as the final or finished dosage form, or as the pharmaceutical composition) are well known and may affect, for example, the solubility, stability, flowability, fractability, and compressibility of drug substances and the safety and efficacy of drug products, (see, *e.g.*, Knapman, K Modern Drug Discoveries, March 2000: 53).

5-Azacytidine (also known as azacitidine and 4-amino-1-β-D-ribofuranosyl-S-triazin-2(1*H*)-one; Nation Service Center designation NSC-102816; CAS Registry Number 320-67-2) has undergone NCI-sponsored trials for the treatment of myelodysplastic syndromes (MDS). *See* Kornblith *et al.*, J. Clin. Oncol. 20(10): 2441-2452 (2002) and Silverman *et al.*, J. Clin. Oncol. 20(10): 2429-2440 (2002). 5-azacytidine may be defined as having a formula of $C_8H_{12}N_4O_5$, a molecular weight of 244.20 and a structure of:



In the United States Patent Application Serial No. 10/390,578 entitled "Forms of 5-azacytidine," filed March 17, 2003 and incorporated herein by reference in its entirety, eight different polymorphic and pseudopolymorphic forms of 5-azacytidine (Forms I-VIII), in addition to an amorphous form, are described. Forms I-VIII each have characteristic X-Ray Powder Diffraction (XRPD) patterns and are easily distinguished from one another using XRPD.

5-azacytidine drug substance used in the previous clinical trials has typically been synthesized from 5-azacytosine and 1,2,3,5,-tetra-O-acetyl-β-D-ribofuranose by the method presented in Example 1. The last step of this method is a recrystallization of the crude synthesis product from a methanol/DMSO co-solvent system. Specifically, the crude synthesis product is dissolved in DMSO (preheated to about 90°C), and then methanol is added to the DMSO solution. The product is collected by vacuum filtration and allowed to air dry.

In In the United States Patent Application Serial No. 10/390,578 entitled "Forms of 5-azacytidine," filed March 17, 2003 and incorporated herein by reference in its entirety, it is demonstrated that this prior art method for the recrystallization of the crude synthesis product does not control for the polymorphic forms of 5-azacytidine. Specifically, the prior art recrystallization procedure produces either Form I substantially free of other forms, or a Form I/II mixed phase *i.e.* a solid material in which 5-azacytidine is present in a mixed phase of both polymorphic Form I and polymorphic Form II. Thus, the prior art procedures do not allow one to reliably target Form I as the single polymorphic form in the drug substance. The present invention provides methods that allow one to recrystallize 5-azacytidine as polymorphic Form I robustly and reproducibly.

## Summary of the Invention

The present invention provides methods for robustly and reproducibly isolating 5-azacytidine as polymorphic Form I substantially free of other forms. The methods involve recrystallizing dissolved 5-azacytidine from a primary solvent/co-solvent mixture and then collecting the resultant crystals. The invention also provides pharmaceutical compositions comprising Form I of 5-azacytidine together with a pharmaceutically acceptable excipient, diluent, or carrier.

## Detailed Description of the Preferred Embodiments

### Polymorphic Form I of 5-azacytidine

Form I of 5-azacytidine is described in United States Patent Application Serial No. 10/390,578 entitled "Forms of 5-azacytidine," filed March 17, 2003 and incorporated herein by reference in its entirety. Table 1 provides the most prominent 2θ angles, d-spacing and relative intensities for Form I observed using X-Ray Powder Diffraction (XRPD) performed according the method of Example 4:

| *2θ Angle (°)* | *d-spacing (Å)* | *Relative Intensity* |
|---|---|---|
| 12.182 | 7.260 | 39.1 |
| 13.024 | 6.792 | 44.1 |
| 14.399 | 6.146 | 31.5 |
| 16.470 | 5.378 | 27.1 |
| 18.627 | 4.760 | 16.0 |
| 19.049 | 4.655 | 35.9 |
| 20.182 | 4.396 | 37.0 |
| 21.329 | 4.162 | 12.4 |
| 23.033 | 3.858 | 100.0 |
| 23.872 | 3.724 | 28.0 |
| 26.863 | 3.316 | 10.8 |
| 27.135 | 3.284 | 51.5 |
| 29.277 | 3.048 | 25.6 |
| 29.591 | 3.016 | 11.5 |
| 30.369 | 2.941 | 10.8 |
| 32.072 | 2.788 | 13.4 |

Table 1: 5-azacytidine Form I - the most prominent 2θ angles, d-spacing and relative intensities (Cu Kα radiation)

Isolation of Polymorphic Form I of 5-azacytidine by Recrystallization

Form I of 5-azacytidine may be reproducibly isolated substantially free of other forms by recrystallizing dissolved 5-azacytidine and collecting the resultant crystals. Specifically, 5-azacytidine is first dissolved completely in at least one suitable primary solvent, preferably a polar solvent, more preferably a polar aprotic solvent. Suitable polar aprotic solvents include, but are not limited to, dimethylformamide (DMF), dimethylacetamide (DMA), dimethylsulfoxide (DMSO), and N-methylpyrrolidinone (NMP). The most preferred polar aprotic solvent is DMSO. Mixtures of two or more primary solvents are also contemplated for dissolving the 5-azacytidine, for example a mixture of DMSO and DMF.

The 5-azacytidine used to form the solution may be synthesized by any procedure known in the art; an exemplary prior art synthesis scheme is provided in Example 1. Any polymorphic or pseudopolymorphic form(s) of 5-azacytidine, including mixed phases, may be used to form the solution. Amorphous 5-azacytidine may also be used to form the solution. It is preferred, but not required, that the primary solvent is preheated to an elevated temperature in order to ensure that the 5-azacytidine is dissolved completely. An especially preferred primary solvent is dimethyl sulfoxide, (DMSO), most preferably preheated to a temperature in the range of about 40°C to about 90°C.

Following solvation of the 5-azacytidine in the primary solvent, at least one co-solvent is added to the solution of 5-azacytidine. Suitable co-solvents include $C_2$-$C_5$ alcohols (which term hereinafter refers to $C_2$-$C_5$ alcohols that are independently: branched or unbranched, substituted or unsubstituted), aliphatic ketones (which term hereinafter refers to aliphatic ketones that are indepedently: branched or unbranched, substituted or unsubstituted), and alkyl cyanides (which term hereinafter refers to alkyl cyanides that are independently: branched or unbranched, substituted or unsubstituted). Preferred $C_2$-$C_5$ alcohols, aliphatic ketones, and alkyl cyanides, along with other suitable solvents, are listed below as Class 2 (solvents to be limited) and Class 3 (solvents of low toxic potential) per the International Conference on Harmonization's (ICH) Guideline for Residual Solvents, July 1997). The use of mixtures of two or more of any of the aforementioned co-solvents is also included within the scope of the invention.

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