EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Assessment report

Dacogen

International non-proprietary name: **decitabine**

Procedure No. **EMEA/H/C/002221**

Assessment Report as adopted by the CHMP with
all information of a commercially confidential nature deleted

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LIST OF ABBREVIATIONS

ADR	adverse drug reaction
AML	acute myeloid leukemia
ANC	absolute neutrophil count
BSA	body surface area
CCO	clinical cut-off
CCO (2009)	DACO-016 clinical cut-off for primary analysis: 28 October 2009
CCO (2010)	DACO-016 clinical cut-off for mature survival and update safety analysis: 29 October 2010
CHMP	Committee for Medicinal Products for Human Use
CI	confidence interval
CL	plasma clearance
CML	chronic myelogenous leukemia
CR	complete remission
CRc	cytogenetic complete remission
CrCL	creatinine clearance
CRi	complete remission with incomplete blood count recovery
CRp	complete remission with incomplete platelet recovery
DMT	disease-modifying therapy
DMC	data monitoring committee
DNMT	DNA methyltransferase
ECOG	Eastern Cooperative Oncology Group
EMA	European Medicines Agency
EORTC	European Organization for Research and Treatment of Cancer
EU	European Union
EFS	event-free survival
FDA	(United States of America) Food and Drug Administration
GCP	Good Clinical Practice
HI	hematologic improvement
HR	hazard ratio
HU	hydroxyurea
ICH	International Conference on Harmonization
ITT	intent-to-treat population
IV	intravenous
IWG	International Working Group
LINE	long interspersed nucleotide elements
MAA	marketing authorization application
MDS	myelodysplastic syndromes
MTD	maximal tolerated dose
NCCN	National Comprehensive Cancer Network
OS	overall survival
PBMC	peripheral blood mononuclear cells
PCH	Pharmachemie B.V.
PD	pharmacodynamics
PFS	progression-free survival
P-gP	P-glycoprotein
PK	pharmacokinetics
Pop PK	population pharmacokinetics
PP	per-protocol population
PR	partial remission
RBC	red blood cell
RFS	relapse-free survival
SAP	statistical analysis plan
SEER	Surveillance, Epidemiology and End Results
SOC	system-organ-class
SOP	standard operating procedure
SPC	Summary of Product Characteristics
SWOG	Southwest Oncology group
TC	treatment choice (with physician's advice)
UK	United Kingdom
US	Unites States of America

Vd_{ss}	volume of distribution at steady state
WBC	white blood cells
WHO	World Health Organization

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Janssen-Cilag International N V submitted on 31 May 2011 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Dacogen, through the centralised procedure falling within the Article 3(1) and point 3 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 28 September 2010.

Dacogen was designated as an orphan medicinal product EU/3/06/370 on 8 June 2006, in the following indication: Treatment of acute myeloid leukaemia.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Dacogen as an orphan medicinal product in the approved indication. The outcome of the COMP review can be found on the Agency's website ema.europa.eu/Find medicine/Rare disease designations.

The applicant applied for the following indication:

for the treatment of adult patients with newly diagnosed de novo or secondary acute myeloid leukaemia (AML), according to the World Health Organisation (WHO) classification

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain tests or studies.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/52/2011 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP [P/52/2011] was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

New active Substance status

The applicant requested the active substance decitabine contained in the above medicinal product to be considered as a new active substance in itself.

Scientific Advice

The applicant received Scientific Advice from the CHMP on 27 April 2006, 20 September 2009. The Scientific Advice pertained to quality, non-clinical and clinical aspects of the dossier.

Licensing status

Dacogen has been given a Marketing Authorisation in 35 countries (including the US) in treatment of patients with myelodysplastic syndromes (MDS)

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: **Pierre Demolis**

Co-Rapporteur: **Ian Hudson**

- The application was received by the EMA on 31 May 2011.
- The procedure started on 22 June 2011.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 9 September 2011 . The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 9 September 2011 .
- During the meeting on 17-20 October 2011, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 20 October 2011 .
- The applicant submitted the responses to the CHMP consolidated List of Questions on 21 March 2012.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 11 May 2012.
- During the CHMP meeting on 21-24 May 2012, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant .
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 15 June 2012.
- During the meeting on 16-19 July 2012, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Dacogen on 19 July 2012.
- The CHMP adopted a report on similarity of Dacogen with Vidaza, Ceplene and Trisenox on 20 October 2011.

2. Scientific discussion

2.1. Introduction

Acute myeloid leukaemia is a clonal disorder caused by malignant transformation of a bone marrow-derived, myeloid stem cell or progenitor cell, which demonstrates arrested maturation and aberrant differentiation. These (myelo)blasts accumulate in the bone marrow, peripheral blood, and other organs such as the central nervous system, lymph nodes, liver, and skin. The effects of uncontrolled, exaggerated growth and accumulation of blasts that fail to function as normal blood cells, and the resultant reduction of normal marrow cells, are anaemia, thrombocytopenia, and neutropenia. The clinical presentation of AML is directly related to ineffective haematopoiesis; patients typically present with signs and symptoms of fatigue, haemorrhage, as well as infections and fever (Löwenberg 1999). Untreated, AML is a rapidly progressing and fatal disease that requires prompt attention (Gilliland 2008).

AML is currently classified following the WHO classification system (Vardiman 2002), which reliably correlates classification with disease outcome.

According to recent guidelines from the European Leukemia Net (Dohner 2010), the British Committee for Standards in Haematology (Milligan 2006), and the AML guidelines from the US NCCN (2011), the management of older patients with AML is guided by performance status, cytogenetics, and the presence of comorbidities (cardiovascular, cerebrovascular, pulmonary, hepatic, or renal dysfunction) which limit the ability of the patient to tolerate cytotoxic (combination) induction chemotherapy.

Standard therapy for patients with AML, aiming to attain a CR and improve survival, is combination induction chemotherapy.

Currently, low-dose cytarabine has been established as an active standard of care for older patients with AML in the EU and US. Palliative therapy with hydroxyurea (HU) or 6-mercaptopurine with supportive care can be administered for white blood cell count control.

Azacitidine (Vidaza) has been approved only for use in high-risk myelodysplasia (MDS), including patients with 20% to 30% bone marrow blasts with multilineage dysplasia that fall into the AML WHO classification. However, this represents a small subset of older patients with AML.

Histamine dihydrochloride (Ceplene) in combination with interleukin-2 is licensed only as maintenance therapy for the treatment of adult patients with AML in first remission.

The subject of this centralised application is decitabine (INN) (ATC code: L01BC08, Antineoplastic and immunomodulating agents, pyrimidine analogues). Decitabine is a cytosine nucleoside analogue which inhibits DNA methyltransferase and is intended for the treatment of Acute Myeloid Leukaemia (AML). Dacogen has been approved in the USA since May 2006 for the treatment of Myelodysplastic Syndrome. An application for marketing authorisation was also submitted to the EMA in 2005, but subsequently withdrawn.

The product is presented as a lyophilised powder, for reconstitution and further dilution prior to administration by intravenous infusion.

2.2. Quality aspects

2.2.1. Introduction

The finished product Dacogen 50 mg Powder for Concentrate for Solution for Infusion is presented as a white, sterile, lyophilised powder. The active substance is decitabine. The excipients used in the concentrate are standard Ph.Eur. excipients: potassium dihydrogen phosphate (buffer), sodium hydroxide (buffer/pH adjustment), water for injection (solvent), hydrochloric acid (pH adjustment). The composition of the product is described in section 6.1 of the SmPC.

The product is intended to be reconstituted with 10 ml of Water for Injections to a solution concentration of 5 mg/ml and then further diluted with intravenous infusion solution (0.9% sodium chloride injection, 5% Dextrose solution or Lactated Ringer's injection) to concentrations of 0.1 mg/ml to 1 mg/ml, prior to administration by intravenous infusion over a 1 hour period.

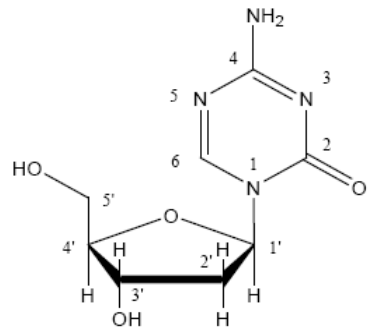
The product is packaged in a clear, Type I glass vial, sealed with a bromobutyl elastomer stopper held in place with an aluminium overseal with a flip-off, plastic (red) cap. Each vial is intended for single use only.

2.2.2. Active Substance

The active substance decitabine (INN) or 4-Amino-1-(2-deoxy-β-D-erythropentofuranosyl)-1,3,5-triazin-2(1H)-one (chemical name) is a white to almost white powder. General physico-chemical properties such as solubility (sparingly soluble in water), pKa values, partition coefficient, hygroscopicity (hygroscopic), stereochemistry (3 chiral centres) and polymorphism (several polymorphs were identified but it was demonstrated that the polymorphs were well-controlled during the process) have been adequately presented.

The active substance is considered by the applicant as a new active substance and is not described in any pharmacopoeia.

The chemical structure of decitabine is as depicted below:



Manufacture

The chemical synthesis of decitabine essentially comprises of three steps using well-defined starting materials and including a purification step. The narrative of the synthesis provided was considered sufficiently detailed; yields and quantities of materials and operating conditions were indicated.

A summary of specifications for all reagents, solvents and processing agents was provided; and it was acceptable. All residual solvents and catalysts are controlled within ICH specifications

Control of critical steps and intermediates were well detailed.

Validation was performed on 3 consecutive, full-scale batches of the active substance. Analytical results were consistent and met the proposed control limits.

The development of the synthesis route was well explained. The impurity profile showed that no impurities above the ICH qualification level of 0.15% could be observed.

The chemical structure and absolute configuration of decitabine has been confirmed by spectroscopy (IR, UV, ^{1}H-NMR, ^{13}C-NMR), high resolution mass spectrometry, elemental analysis, optical rotation and single crystal x-ray crystallography. Physical characterisation was performed using powder X-ray diffraction, differential scanning calorimetry, thermo-gravimetric analysis, dynamic vapour sorption, hot stage microscopy and infrared spectroscopy. The established structure is shown to be consistent with the analytical and spectroscopic data and is stated to comply with that obtained from published literature. The control limits for specific optical rotation are accepted as adequate to assure stereoisomeric purity on a routine basis.

Polymorphism was controlled during the manufacturing process and since the product is reconstituted as a solution and diluted before administration the polymorphic form is not considered as a critical attribute for the active substance.

An extensive and satisfactory discussion was presented on the impurities including the potential genotoxic impurities. The active substance itself, a structural analogue of the DNA building block, 2′-deoxycytidine, has been shown to be genotoxic. Structure-based assessment (DEREK) has been used to identify potential genotoxins among the starting materials, intermediates, reagents, and impurities of the active substance. Given the nature of the active substance, this assessment focused on unique alert structures, unrelated to the structure of the active substance. Those structures which triggered an alert owing to the nucleoside structure shared with the parent molecule were considered to be qualified by decitabine itself. This approach is accepted and the impurities were found below toxicological qualification limits. The tresholds were in line with the ICH Q3A guideline.

For the residual solvents, the proposed limits meet or are more stringent than those recommended by ICH Q3C guidance. A suitable GC method has been developed and validated to monitor residual solvent content. The batch analyses were in compliance with the limits of the ICH Q3C guideline.

Specification

Appropriate specification applied to the active substance included: appearance (visual), identification (IR and HPLC), clarity of solution (Ph.Eur.), specific rotation (Ph. Eur.), assay of decitabine (HPLC), chromatographic purity (HPLC), residual solvents (GC), water content (Ph.Eur. 2.5.32), residue on

ignition (Ph.Eur. 2.4.14), particle size (laser diffraction), heavy metals (USP, harmonised with Ph.Eur.), microbiological quality (Ph.Eur. 2.6.12 and Ph.Eur. 2.6.13) and bacterial endotoxins (Ph.Eur. 2.6.14).

The Analytical methods used have been adequately described and the in-house methods have been adequately validated.

Batch analysis of 4 batches reflecting a variety of sources, process scales and synthetic routes were presented and all data complied with the proposed specification.

The specification for the active substance are adequately justified and set according to ICH guidelines and Ph. Eur. requirements.

The container closure system consists of double polyethylene bags placed within an opaque, HDPE drum. Silica gel is used as a desiccant. The primary packaging conforms with the requirements of EU Directive 2002/72/EC. A suitable specification has been provided along with the IR spectrum.

Stability

Stability studies were conducted on three commercial batches of decitabine under ICH conditions: at 30°C/65%RH (up to 18 month data) and at 40°C/75%RH (6 months data).

The selected stability parameters comprise appearance and clarity of solution, water content, specific rotation, assay and related substances. Polymorphism does not affect the final product therefore this parameter was not assessed during stability

Under all storage conditions, minor variability but no significant changes were observed for any parameter and the substance complied with the proposed specifications.

In addition, a photostability study was performed in line with ICH recommendations for exposure to UV and visible light. All test parameters above, supplemented by tests for microbial purity and bacterial endotoxins satisfactorily demonstrated the photostability of decitabine after 4 weeks of ICH recommended light exposure. Forced degradation studies (acidic, basic, oxidation, and high temperature) were conducted on one batch of decitabine (solid state and solution). The results showed that decitabine was stable under oxidation and high temperature but was unstable under alkaline conditions.

Based on these data, the proposed re-test period was accepted, when packaged in the proposed container and under no special temperature storage conditions.

2.2.3. Finished Medicinal Product

Pharmaceutical Development

Components of the Drug Product

Decitabine is a white to almost white powder. Solubility and particle size were discussed, and although particle size was not critical for the solubility it was controlled to ensure good manufacturability. Once processed, the lyophilized product is reconstituted with water for injection and further diluted for

infusion, at which point active substance particle size is not relevant. Polymorphism is well controlled during the manufacturing process.

The finished product is formulated with compendial and commonly used pharmaceutical excipients. The function of each excipient was well described. Excipients used were mainly for pH adjusting and buffering agents. Nitrogen is a processing aid used during the lyophilization process to provide an inert atmosphere for reducing moisture content. Stability data for the drug product were considered acceptable to demonstrate the absence of incompatibility between the active substance and the excipients.

Formulation Development

Since decitabine is heat sensitive and prone to hydrolysis in aqueous solution, it was justified to use a lyophilised powder for reconstitution at the time of use. To optimise stability in aqueous solution, critical parameters such as the pH and temperature were controlled.

The parenteral route of administration was preferred due to its maximum bioavailability, decitabine being degraded in the GI tract.

Overages

No overages are applied to this product.

Physicochemical and Biological Properties

This section addressed the key physicochemical attributes related to drug product performance. Since Decitabine is hygroscopic and degrades in aqueous solutions, minimizing the moisture content of the finished product by lyophilization ensures a stable product. The amount of time in aqueous solution during the compounding, filling, and lyophilization of the finished product was also carefully controlled to minimize the rate of degradation. The selected container/closure system provided an adequate moisture barrier as demonstrated by long-term stability data. The pH of the aqueous solution and the duration in solution during the compounding, filling, and lyophilization of the finished product were carefully controlled to minimise the rate of degradation.

Manufacturing Process Development

The heat sensitivity of decitabine in aqueous solution was acknowledged from in-use stability data. Thus, the use of aseptic filtration as a means of sterilisation is accepted without additional confirmation. Process development, including minor changes implemented on moving from pilot scale for Phase I clinical and registration lots to the current production scale were outlined and acceptable.

Justification of critical process parameters such as mixing time, pH, temperature, and sterile filtration was provided.

The bulk solution holding time was well established as it is critical to minimise degradation. Development and optimisation of the lyophilisation cycle was adequately described.

The pharmaceutical development of the finished product contains Quality by design (QbD) elements.

For each unit operation, a summary of the studies conducted at development, pilot and/or commercial scale was presented. The lyophilization cycle parameters have been optimized and well characterized during the development and scale-up studies. The information provided was satisfactory.

The proposed proven acceptable ranges for process parameters are typical operating ranges were considered acceptable. The pH of the aqueous solution and the duration of the active substance in solution during the compounding, filling, and lyophilization of the finished product were carefully controlled to minimise the rate of degradation

The rationale for the critical parameters identified such as pH was satisfactorily provided.

All clinical batches have been manufactured at industrial scale according to the commercial formulation. All of them used active substance from a previous supplier including pivotal phase 3 study (DACO-016) batches. Taking into account that particle size distribution and impurity profile have been compared and found similar between the two decitabine sources (current applied manufacturer and previous manufacturer for the clinical batches) on a significant number of batches, and that confirmation batches have demonstrated that the commercial manufacturing process leads to finished product with the same satisfactory characteristics, clinical batches are thus considered representative of the proposed commercial product.

Container Closure System

The proposed container closure system (glass vial with elastomer stopper / aluminium crimp seal) was typical for lyophilised parenteral products and conform to Ph. Eur. type I standards including closure integrity. The compatibility of the packaging with drug product was demonstrated by the stability data.

Microbiological Attributes

The manufacturing process including sterile filtration of the bulk solution and aseptic processing, has been correctly validated. Sterility and bacterial endotoxins testing were included in the release and shelf life specifications ensuring the compliance of the finished product with compendial requirements. The integrity of all container closure systems has been demonstrated by maintenance of sterility.

Compatibility

The finished product is reconstituted with Water for Injections prior to further dilution with infusion fluid.

Decitabine undergoes hydrolytic degradation in aqueous solutions. Compatibility stability studies were performed: in-use stability of the reconstituted and diluted solution and stability of diluted solution delivered through infusion sets, both at room temperature and refrigerated conditions. Data confirmed the compatibility and the stability for the reconstituted solution and the diluted infusions as described in the SmPC.

Adventitious agents

None of the excipients used were of human or animal origin. Appropriate declarations were included in section 3.2.R.4. No TSE risk was anticipated.

Manufacture of the product

In summary, the manufacturing process consists of dissolution of the excipients and active substance in Water for Injections, pH adjustment followed by aseptic filtration, filling and lyophilisation. A detailed flow diagram of the manufacturing process was provided, highlighting in-process controls at the relevant stages such as pH control, filter integrity.

Process Validation

Given the reliance upon aseptic processing for achieving and maintaining sterilisation, this process was considered to be non-standard.

In line with the process validation guideline (CPMP/QWP/848/96), process validation data at the proposed commercial scale were presented for three batches of finished product.

Validation data included an evaluation of process parameters, in-process controls such as compounding, sterile filtration and filling and analysis to the proposed finished product specification. This approach was found acceptable and should ensure that the manufacturing process leads to a product of consistent quality.

Specifications

All excipients used in the proposed finished product were in compliance with the relevant Ph. Eur. monograph. No validation was deemed necessary for compendial analytical procedures used for the analysis of Ph. Eur. excipients. The specifications for the Ph. Eur. excipients are in line with the Ph. Eur. monographs and no further justification is necessary.

Novel Excipients

No novel excipients were used in the manufacture of the finished product.

Product specification

The finished product release specification included the following parameters: appearance (visual), identification tests (UV Ph.Eur 2.2.25 and HPLC), reconstituted solution, clarity of solution (Ph.Eur. 2.2.1), visible particles (Ph.Eur. 2.9.20), subvisible particles (Ph.Eur.2.9.19), pH (Ph.Eur. 2.2.3), assay (HPLC), chromatographic purity (related substances, HPLC), uniformity of dosage units (Ph.Eur. 2.9.40), water content (Karl Fisher), sterility (Ph.Eur. 2.6.1), bacterial endotoxins (Ph.Eur. 2.6.14).

The shelf-life specifications included only the following parameters: appearance (visual), reconstituted solution, clarity of solution (Ph.Eur. 2.2.1), visible particles (Ph.Eur. 2.9.20), subvisible particles

(Ph.Eur.2.9.19), pH (Ph.Eur. 2.2.3), assay (HPLC), chromatographic purity (related substances, HPLC), water content (Karl Fisher), sterility (Ph.Eur. 2.6.1), bacterial endotoxins (Ph.Eur. 2.6.14).

Specifications were well justified and the limits applied to specified impurities were below the qualified levels.

Analytical Procedures

The general methodology of the Ph. Eur. was referenced for identification by UV, clarity of solution, visible and sub-visible particles, pH, uniformity of dosage units (by mass variation), sterility and bacterial endotoxins. In-house methods are described for appearance, identification by HPLC retention time, reconstitution time, assay, related substances and water content by Karl Fischer. Validation of the in-house analytical methods has been adequately performed.

Batch Analyses

Batch analysis data were presented for five production-scale batches. These were supplemented by four registration / validation lots and six clinical lots. Additional data generated at a smaller batch scale were also presented. All data were satisfactory and in compliance with the proposed specifications.

Stability of the product

Stability studies were conducted on six batches (pilot and production-scale) of the finished product packed in the commercial packaging under ICH conditions: 2-8 °C (1 batch for 24 months), 25 °C / 60% RH (7 batches up to 36months), 30 °C / 75% RH (1 batch for 24 months), 40 °C / 75% RH (6 batches for 6 months). The active substance was obtained from the different manufacturers used.

The tests performed were: appearance, reconstitution of solution (time, clarity, visible particles), assay, related substances, pH, water, closure integrity, sterility, and bacterial endotoxins. The analytical methods were stability indicating and were the same as those used for the release product testing.

Over the stability period, product appearance, visible particles and clarity of reconstituted solution continued to meet specification.

Under the long term conditions, no significant change could be observed in the test parameters, and under higher temperature the results remained also within the specification.

Photostability data generated in line with ICH guidance were provided for a single lot in primary packaging and confirm the photostability of the active substance.

Overall, the data support the proposed shelf-life and the storage conditions as proposed in the SmPC.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance decitabine and the finished product lyophilised Powder for Concentrate for Solution for Infusion has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality product characteristics, and these in turn lead to the conclusion that the product should a satisfactory and uniform performance in the clinic.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of Dacogen is satisfactory when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled appropriately. Data has also been presented regarding TSE and no concern was identified.

2.3. Non-clinical aspects

2.3.1. Introduction

Decitabine belongs to the deoxycytidine analogues. It is a prodrug that requires metabolic activation by deoxycytidine kinase. Indeed, prior to the incorporation of decitabine into the DNA, the drug has to be phosphorylated by several kinases. Decitabine triphosphate is the active moiety which is incorporated into DNA.

Decitabine produces antitumor effects by specifically targeting DNA methyltransferases (DNMTs) that maintain the epigenetic silencing of tumor suppressor genes and contribute to the progression of cancers. Decitabine, a cytidine deoxynucleoside analogue, is specifically incorporated into DNA leading to the covalent trapping of DNMTs in a complex with the decitabine substituted DNA. This depletes the DNMT activity in cells, leading to DNA hypomethylation in gene promotor regions. This results in re-expression of silenced genes that can inhibit cellular proliferation, induce differentiation or induce apoptosis.

2.3.2. Pharmacology

Primary pharmacodynamic studies

In vitro studies

General antiproliferative effects

- Leukemia models

The *in vitro* activity of decitabine was measured in several human and murine models (see Table 1).

Table 1: *In vitro* activity of decitabine against animal and human tumor cells.

Cell type	IC50 (µM)	References
L1210 (murine)	0.01	Momparler, 1984b
HL-60 promyelocytic leukemia	0.1 to 1.0	Momparler, 1985a; Leyva, 1986; Yang, 2004 ; Qin, 2007 ; Qin, 2009
CEM T-cell leukemia	1.0	Antonsson, 1987
MOLT-4 T-cell leukemia	1.8	Qin, 2009
RPMI 8226 EBV- myeloma	0.021	Lavelle, 2003
ARH-77 EBV+ lymphoblastic cells	0.049	Lavelle, 2003
HS-Sultan EBV+ lymphoblastic cells	0.026	Lavelle, 2003
RAJI lymphoid leukemia	0.054	Qin, 2007 ; Qin 2009
TF-1 leukemia	0.01	Qin, 2009
HAL leukemia	0.04	Qin, 2009
ML-1 leukemia	0.098	Qin, 2007 ; Qin, 2009
K562 leukemia	0.4	Qin, 2009
Jurkat leukemia	1.0 – 2	Qin 2007 ; Qin, 2009
Primary culture AML	About 0.25	Motoji, 1985
OPM-2 EBV- myeloma	0.036	Lavelle, 2003
Primary culture CML	0.04-0.4	Limonta, 1993

Decitabine was also shown to induce morphological and functional differentiation in leukemia cell lines (see Table 2).

Table 2: Decitabine induction of differentiation in murine and human leukaemia cell lines

Cell type	Concentration (µM)	Exposure time (hr)	Differentiation (%)	References
10T1/2 tumor	1.0	54	14	Momparler, 1985b
Erythroleukemia	0.25	24	15	
Myeloid leukaemia	1.0	72	54	
HL-30	0.2	48	30	

In the OCI-AML2 and KG1 human AML cell lines decitabine had antiproliferative and cytotoxic effects. A dose- and time-dependent inhibition of proliferation was observed following single pulse treatments with 1.0 and 5.0 µM. Analysis of DNA fragmentation indicated significant pro-apoptotic effects (Schmelz, 2005b).

In vitro, it seems that high doses of decitabine induce cytotoxicity while low doses induce hypomethylation. Indeed, it was demonstrated that the dose-dependant curve representing methylation due to decitabine treatment has a U-shape. It appears that at concentrations higher than 0.5 or 1.0 µM, methylation increases (Qin, 2007; Qin 2009).

Similarly, in the human lymphoid CCRF/CEM/0 line, decitabine decreased the methylation level to 47% of baseline at 0.3 µM, while the IC_{50} for cell survival attained 1 µM, suggesting a gap between the hypomethylating doses and the cytotoxic doses (Antonsson, 1987).

- Solid tumor

The activity of decitabine was assessed in different solid tumor models (breast, colon, prostate cancer). IC50 values varied from nM to the µM range (Qin, 2009).

In HT-29 human colon cancer cells, the concentration of 1 µM of decitabine produced a 90% inhibition of methylation without causing cell lethality (Glazer, 1984).

Decitabine resistance

Phosphorylation by deoxycytidine kinase represents the initial step to decitabine activation prior to incorporation into DNA. Complete loss or reduction of deoxycytidine kinase activity results in resistance to decitabine (Vesely, 1970).

Uridine kinase activity was also reduced in decitabine resistant tumors (a 10% decrease) but the decrease was more important in 5-azacytidine resistant tumors (decrease of 74%). The deletion of both kinases could explain cross-resistance of 5-azacytidine-resistant leukemic cells to decitabine (Vesely, 1970).

Intracellular pools of deoxycytidine triphosphate (dCTP) can also alter the sensitivity of cells to decitabine. Reduction of dCTP pools by treatment of the L1210 leukemia and EMT6 murine breast cancer cell lines with 3-deazauridine (3-DU), an inhibitor of cytidine triphosphate (CTP) synthetase that depletes intracellular pools of CTP and dCTP, increased the antiproliferative activity of decitabine *in vitro* by increasing the incorporation of decitabine into DNA (Momparler, 1979b). Pools of intracellulars dCTP can also be reduced by high doses of exogenous thymidine (Grant, 1982).

Deamination of decitabine by cytidine deaminase may also play a role in mediating lack of response to decitabine. Cytidine deaminase is a key enzyme in the metabolism of cytosine nucleoside analogues since their deamination is a major pathway of pharmacokinetic disposition of these agents resulting in a complete loss of antileukemic activity. It has been shown that treatment with decitabine is associated with an increase in cytidine deaminase activity in decitabine-resistant HL-60 cells and in leukemic cells of some decitabine-treated patients (Momparler, 1990). Additionally, the intracellular phosphorylated decitabine intermediates may also be deaminated by deoxycytidylate deaminase (Momparler, 1984a).

In order to evaluate the interaction of decitabine with the P-glycoprotein efflux pump, bi-directional permeability of decitabine through MDR1 transfected MDCK cell monolayers was studied. Results from these *in vitro* experiments indicated that decitabine is a poor P-gp substrate and therefore is not susceptible to multi-drug resistance mediated by P-gp.

In a panel of cancer cell lines, it was demonstrated that sensitivity to decitabine showed a low correlation to that of 5-azacytidine (AZA), but a good correlation to that of cytarabine. The most resistant cell lines to decitabine had a combination of low deoxycytidine kinase, hENT1 and hENT2 nucleoside transporters and high cytosine deaminase (Qin, 2009). However, the in vitro drug sensitivity does not always correlate with drug resistance in patient (Momparler, 1985c).

In vivo studies

The antitumor activity of decitabine alone or in combination has been studied in several leukaemia models.

Non-comparative studies

Decitabine anti-tumor activity was tested in several mouse leukaemia models (AKR, P388, L1210) (see Table 3).

Table 3: *In vivo* activity of decitabine in mouse leukemia models

Tumor Implant	Drug Route	Total dose (mg/kg)	Schedule	T/C (%)[a]	Reference
AKR					
i.p.	i.p.	0.25	Day 1-5 (5 doses)	192	Vesely, 1977
		5.0		203	
		7.5		252	
		12		148	
		20		102	
P388					
i.p.	i.p.	1	Day 1-5 (5 doses)	140	Vesely, 1977
		2		160	
		4		255	
		8		250	
		16		90	
		32		70	
L1210					
i.p.	i.p.	1	Day 1-5 (5 doses)	186	Vesely, 1977
		2		179	
		4		136	
		8		114	
		16		129	
		32		114	
i.p.	i.p.	100	Day 3	267	Colombo, 1986
		100	Day 5	188	
		150	Day 3, 5 and 9	311	
i.p.	i.v.	50	Day 3	312	Zaharko, 1984
		100	Day 3 and Day 10	387	
		100	Day 3 and Day 17	412	
		150	Day 3, 10 and 17	450	
i.v.	i.v.	3.2	9-h infusion on D1	207	Momparler, 1989
		12.4		345	
i.v.	i.v.	0.004	8-h infusion on D3	101	Momparler, 1978
		0.016		109	
		0.44		119	
		4.4		177	
		42.9		372	
i.v.	i.v.	0.56	15-h infusion on D1	129	Wilson, 1983
		2.3		180	
		5.5		254	
		21.3		No death[b]	
i.v.	i.v.	0.97	6-h infusion on D3	132	Momparler, 1979b
i.v.	i.v.	9.8	12-h infusion on D3	318	
i.c. [c]	i.v.	1	12-h infusion on D6	104	Chabot, 1984
		5		143	
		20		222	
		30		213	
		45		215	
		67		221	
i.c. [c]	i.v.	20	12-h infusion on D3	246	
		20	12-h infusion on D4	216	
		20	12-h infusion on D5	244	

[a] Test/control survival time expressed as percentage with control = 100%
[b] 10 animals/10 survived at the end of the study (i.e. 40 days)
[c] i.c. = intra-cerebral

Decitabine was also tested in a rat myeloid leukaemia model, the Brown Norway rat leukaemia model (BNML). A dose-response relationship was demonstrated for doses up to 50 mg/kg (3 times q 12h), a higher dose resulted in only a slight increase in median survival time.

Comparative studies

Studies were performed to compare the activity of decitabine with cytarabine and azacytosine in mouse leukaemia L1210. When mice were treated with increasing doses of each agent up to a maximal tolerated dose, decitabine was the most effective compound. Decitabine was the only drug that allowed all the treated animals to survive more than 60 days (see Table 4) (Momparler, 1984b).

Table 4: Effect of Decitabine, Cytarabine and Azacytosine against L1210 Mouse Leukemia

Drug	Total dose (mg/kg)	Survival time (days)	ILS [a] (%)	60 days survivors [b]	Lethal drug toxicity [c]	Weight change day 7 (%)
none	-	6.1 ± 0.5*	0	0/10	-	-
Decitabine	5.0	24.6 ± 4.4	297	0/5	0/5	+1
	10.1	30.0 ±6.7	384	0/5	0/5	-5
	20.6	48.0 ± 2.5	674	3/5	0/5	-13
	41.4	> 60	-	5/5	0/5	-24
Cytarabine	468.9	20.6 ± 5.3	232	0/5	0/5	-13
	969.4	25.4 ± 5.3	310	0/5	0/5	-15
	1442	21.6 ± 2.3	248	0/5	0/5	-18
	1995	30.3 ± 5.5	389	0/5	2/5	-22
Azacytosine	11.7	10.1 ± 2.2	63	0/5	0/5	-4
	24.1	13.3 ± 1.1	115	0/5	0/5	-3
	48.9	17.4	181	0/5	2/5	-10

CD2F1 male mice were injected i.v. with 105 L1210 leukemic cells on day 0 and treated on day 1 with 15 h i.v. infusion
*Mean ± standard deviation
[a] ILS: Increased Life Span
[b] Number of mice/total. Mice that survived > 60 days were not included in the ILS calculation
[c] Number of mice/total. Mice that survived < 10 days were not included in the ILS calculation

In the Brown Norway rat leukemia model, decitabine activity was compared with cytarabine. At the maximum dose of decitabine (250 mg/kg) and cytarabine (1000 mg/kg) tested, decitabine induced a 10-day longer survival time than cytarabine which means 2 logs more of leukemic cell kill for decitabine (Richel, 1988).

Combination studies

- Combination studies with Histone Deacetylase

Histone acetylation has been shown to work in concert with methylation to regulate gene transcription. Acetylation of histone is required to maintain chromatin in an open and transcriptionnally active state. This permits binding of transcriptional factors, histone acetylases and other regulatory co-activators that promote gene expression. Conversely, histone deacetylases (HDAC) act to keep these residues deacetylated and thus maintain transcriptional silencing. Also, binding of HDAC to hypermethylated chromatin, under DNMTs direction produces a DNA binding complex capable of blocking the access of the transcriptional activators to promoters. It should be noted that methylation is dominant to histone deacetylation, so transcription cannot occur without first inhibiting methylation. Initial treatment with DNMT inhibitors followed by HDAC inhibitors can produce additive or synergistic effects for re-expression of transcriptionnally silenced genes (Baylin, 2005).

In *in vitro* studies, a synergistic induction of apoptosis was observed when the pan-HDAC inhibitor JNJ-26481585 (12.5 to 250 nM) was combined with decitabine (1 µM) pretreatment in several leukemic cell lines (U937,HL60, THP1 and MOLT4). In U937 and THP1 cells, significant induction of H3 acetylation was observed after pre-treatment with decitabine followed by JNJ-26481585, compared with single agent JNJ-26481585 (Tong, 2010).

The *in vitro* treatment of decitabine and phenylbutyrate, a histone deacetylase inhibitor, in combination produced a greater inhibition of growth, DNA synthesis and a greater reduction on colony formation on both L1210 and human HL-60 leukemic cells as compared to either drug alone. The combination also produced a synergistic activation of the tumor suppressor gene p15CDN2B in the L1210 cells. In mice with L1210 leukemia, the combination showed enhanced antineoplastic activity (Lemaire, 2004).

The activity of the combination of decitabine and the HDAC inhibitor valproic acid (VPA) was studied in two different leukemia cell lines: HL-60 and MOLT4. When combined, decitabine and VPA had an additive effect on leukemia cell kill and apoptosis, as well as on the reactivation of p57KIP2 and p21CIP1 (Yang, 2004).

The combination of decitabine with trichostatin or depsipeptide FR901228, two potent inhibitors of histone deacetylase, produced a greater inhibition of growth and DNA synthesis and a greater loss of clonogenicity than either agent alone in HL60 and KG1a leukaemia cell lines (Shaker, 2003).

- Combination with antimetabolites and drugs used to treat leukaemia

Cytarabine is the most active agent in myeloid leukemia. Their combination was studied in different human leukemia cell lines. The combination of decitabine and ara-C showed additive induction of cell death in ML-1 and synergistic induction in HL60, Raji, and Jurkat. Sequentially, decitabine followed by cytarabine was a synergistic combination in all cell lines. But antagonism was observed in terms of epigenetic effects (cytarabine reduced the effects of decitabine on hypometylation and gene re-expression). However, it is likely that the antagonism is apparent but not real and reflects killing of the most hypomethylated cells (Qin, 2007).

In vivo, cytarabine given simultaneously with decitabine to L1210 leukemic mice strongly reduces decitabine activity against L1210 leukemia (T/C was 267% and 156% for a single treatment on Day 3 and 311% and 211% for a 3-time treatment on Day 3, 6 and 9 for decitabine and the combination decitabine+cytarabine respectively). This can be explained by the fact that cytarabine prevents decitabine incorporation into DNA (Colombo, 1986).

The combined effects of decitabine and hydroxyurea, a ribonuclease reductase inhibitor used in the treatment of leukemias and sickle-cell disease, were tested in HL-60 and T24 cell lines *in vitro*. The concomitant treatment of hydroxyurea with decitabine resulted in a reduction of the inhibition of DNA methylation induced by decitabine. The reduction in decitabine efficacy was attributed to the arrest of the cell cycle induced by hydroxyurea. By contrast, sequential exposure to the two drugs produced a synergistic effect (Choi, 2007).

Secondary pharmacodynamic studies

Modulation of the haemoglobin gene

Fetal hemoglobin (HbF) decreases polymerization of sickle haemoglobin (HbS) and the complications of sickle cell disease (SSD). Therefore, a therapeutic goal in SSD is pharmacologic reactivation of HbF.

Silencing of the γ-globin (HbF) gene is associated with DNA methylation. Symptomatic SSD patients were treated with decitabine. γ- Globin gene promoter methylation decreased. Treatment decreased neutrophils and increased mean HbF and mean total hemoglobin. Features of vaso-occlusive crisis pathophysiology such as red cell adhesion, endothelial damage, and coagulation pathway activity significantly improved (Sauntharajah, 2003). A study in baboons confirmed that decitabine increases fetal hemoglobin in vivo by transcriptional activation of the γ -Globin gene (Akpan, 2010).

Effects on normal hematopoietic cells

In an *in vitro* model of normal hematopoietic differentiation, using CD34+ cells from mobilized peripheral blood, decitabine treatment induced a dose-dependant decrease of cell-growth without causing cytotoxicity. It also increased the expression of different markers (lysosomes, myeloperoxidase, CD15) suggesting an induction of differentiation (Guo, 2006).

Pharmacology of the degradants and α-enantiomer

In aqueous solution, decitabine can degrade into several molecules. Some of the degradants were evaluated in a toxicity study.

Decitabine can also isomerise into the α-anomer, which involves the orientation of the deoxyribose moiety. The α-anomer was much less active as an antiproliferative agent *in vitro* in L1210 leukemia cells. It was about 100-fold less active (as shown by the increased life span) in L1210 leukemic mice after a single i.p. administration and 100-less toxic after daily i.p. administration for 1 week in mice (Vesely, 1984).

In human lymphoblastoid CCRF-CEM cells, the α-anomer induced hypomethylation of specific DNA loci at concentrations comparable to decitabine while cytotoxicity of the α-anomer was seen at 4-fold higher concentrations compared to decitabine (IC50 = 2.4 and 0.6 µM, respectively). Since α-anomers of nucleosides are generally not incorporated into DNA, it is possible that the α-anomer activity is due to its conversion into the ß-anomer (decitabine) (Fojtova, 2007).

Exposure to the α-anomer induced the down-regulation of hTERT gene expression at lower concentrations than decitabine that was more cytotoxic (Hajek, 2008).

Safety pharmacology programme

Effects on central nervous system

No adverse behavioural effects, neurological impairment or adverse autonomic responses were reported in an Irwin Test in which male mice were dosed with a single i.v. bolus injection of up to 150 mg/m2 (50 mg/kg) and plasma decitabine levels greatly exceeding clinical plasma levels (mean Cmax 137 ng/mL). At higher doses tested in general toxicity studies, ataxia and convulsions characterized acute toxicity and were clearly related to decitabine Cmax.

Effects on cardiovascular and respiratory system

In vitro, decitabine had no significant effect on the membrane K+ current (IKr) in hERG-transfected HEK293 cells up to a concentration of 30 µM (6.8 µg/mL), a concentration that greatly exceeds clinical drug plasma levels. This in vitro assay was not performed under GLP.

The effects of decitabine on cardiovascular and respiratory function were investigated in vivo in the conscious, telemetered male and female cynomolgus monkey, which received a single 1-hour i.v.

infusion of 628.8 mg/m2 (52.4 mg/kg). The cynomolgus monkey was used to allow for an adequate evaluation of the cardiovascular parameters by telemetry in a relevant non-rodent species. Decitabine had no effects on diastolic or systolic arterial blood pressure, lead II electrocardiogram (ECG) variables (PR, RR, QT, QTcB and QTcF intervals, and QRS complex), ECG gross morphology (rhythm and waveform) or (electromyography-derived) respiratory parameters. A decrease in heart rate in vehicle (0.9% NaCl) and decitabine treated animals, in comparison with baseline values, was slightly greater and variable in decitabine dosed animals, but did not result in a statistically significant difference in heart rate at any time point between the vehicle and decitabine groups and is therefore considered of no relevance. Decitabine plasma exposure, based on a separate single dose i.v. 1-h infusion monkey study at the same dose level indicated that the exposure in monkeys was 20 to 30-fold higher than the clinical exposure.

Pharmacodynamic drug interactions

No specific pharmacodynamic drug interaction studies with decitabine were performed.

2.3.3. Pharmacokinetics

Pharmacokinetics of decitabine was studied in mice, rats, dogs, rabbits and monkeys. The profile was roughly the same in every species. Decitabine is unstable in aqueous solution and in plasma in *in vivo* conditions (37°C, pH 7.4).

Absorption

After an i.v. single administration, decitabine plasma levels declined bi-exponentially in every tested species, with a fast initial decline (few minutes) and a somewhat longer elimination half-life (23 minutes in mice, 44 minutes in rabbits, 1 hour in monkey, 69 to 144 minutes in dogs and 3 hours in rats). This species difference in the elimination of decitabine was also found in the clearance values. The clearance of decitabine was highest in the monkey, followed by the mouse and the rabbit and then the dog and the rat. This species difference is consistent with the species differences in the activity of cytidine deaminase, the main metabolizing enzyme of decitabine.

Decitabine showed a low to moderate oral bioavailability. It was 10% in mice, 57-67% in juvenile rats, 35% in rabbits and 2% in monkeys.

In monkeys, food had a low influence on exposure.

Repeated administration in rats and rabbits did not dramatically alter the pharmacokinetic profile.

Distribution

Following i.v. administration, the apparent volume of distribution at steady state (Vdss) was about 1.1 L/kg in mice, about 1.8 L/kg in rats, about 0.8 L/kg in rabbits, about 1.0 L/kg in dogs and 4.3 L/kg in monkeys.

In the QWBA study, the bi-exponential elimination of decitabine was confirmed. An initial rapid decline of total radioactivity was followed by a more gradual decrease. Distribution was wide and rapid. The highest radioactivity levels by far were found in the lymphatic system (bone marrow, thymus and spleen) supporting the indication of decitabine in leukemia.

Decitabine was also demonstrated to be able to penetrate the cerebrospinal fluid.

At physiological pH, protein binding is very low, <1%.
Decitabine appeared to be neither a substrate nor an inhibitor of P-gp.

Decitabine influx is mediated by hENT1 and other members of the nucleoside transporter family.

Metabolism

The comparison of *in vitro* metabolic profiles of different species showed that the mouse, the rabbit and the monkey were the closest species to humans. All the human metabolites were not present in rats and dogs.

In vivo studies in rats and rabbits demonstrated that mice metabolised decitabine more extensively than humans and rabbits. All the human metabolites were qualitatively covered by mouse and rabbit metabolites (except for M6 absent in rabbit).

The primary metabolic pathways were oxidative deamination (probably mediated by cytidine deaminase), oxidation, hydrolysis and deformylation.

The unidentified polar metabolites M1 and M2 were the major metabolites in human plasma and urine whereas they were much less abundant in animals.

In rabbits, M3 that resulted from decitabine oxidation was the major metabolite (30%). In mice, M4 that resulted from decitabine oxidative deamination and deformylation was the major metabolite (25%) and degradation products represented 10% of the urine metabolites. These degradation products were not observed in rabbits and humans.

Decitabine is subjected to enzymatic activation and inactivation. The main inactivation pathway is mediated by cytidine deaminase that has a 20 times higher affinity to its natural substrates than to decitabine. Its degradation is also mediated dCMP deaminase that transforms decitabine monophosphate to 5-aza-dUMP. Cytochrome P450-mediated degradation is minor.

The phosphorylation of decitabine is essential before its incorporation into DNA. The enzyme responsible for its activation is deoxycytidine kinase.

Decitabine did not inhibit CYP1A2, CYP2C8, CYP2C19, CYP2D6, CYP3A4 and CYP2C9 or induce CYP1A2, CYP2B6, CYP2C9 and CYP3A4/5 at clinically relevant concentrations.

Decitabine treatment could induce cytidine deaminase activity at 0.1 µM or higher (22.8 ng/mL). This could be relevant in the evaluation of the clinical response of patients.

Excretion

In mice and rabbits, decitabine is rapidly excreted into urine which is the main excretion route (83.4 and 66.7% respectively). Feces excretion was minor (about 8% in both species).

Drug-drug interaction

The metabolic pathway of decitabine can lead to drug-drug interactions. Drugs that can interfere with activating (deoxycytidine kinase) or deactivating enzymes (cytidine deaminase) involved in the metabolism of decitabine can change the plasma concentration of the drug.

2.3.4. Toxicology

Single dose toxicity

Single dose toxicity studies were conducted in mice, dogs and monkeys (see Table below).

Table 5: Summary of single dose toxicity studies

Study reference / GLP compliance	Species / Sex / Number / Group	Dose (mg/kg) / Route	Observed max non-lethal dose (mg/kg)	Approx. lethal dose (mg/kg)	Major findings
079379 GLP	Swiss Mouse 10/sex/group	65, 85, 115, 150, 200 i.v. (bolus)	115 (M) 150 (F)	150 (M) 200 (F) LD50 = 191 (M) 207 (F)	Males **65 mg/kg**: piloerection **115 mg/kg**: piloerection, swelling mandible **150 mg/kg**: piloerection, mortality (1/10) **200 mg/kg**: mortality (6/10) Females **150 mg/kg**: piloerection **200 mg/kg**: piloerection, swelling mandible, snout and throat area, Mortality (2/10)
079392 GLP	Swiss Mouse 10/sex/group	85, 115, 150, 200, 250 i.v. (bolus)	115 (M) 150 (F)	150 (M) 200 (F) LD50 = 215 (M) 224 (F)	Males **85 mg/kg**: piloerection, swollen abdomen **150 mg/kg**: piloerection, mortality (1/10) **200 mg/kg**: piloerection, mortality (4/10) **250 mg/kg**: lethargy, piloerection, mortality (7/10) Females **150 mg/kg**: swelling toe **200 mg/kg**: piloerection, mortality (1/10) **250 mg/kg**: mortality (9/10)
TOX8653 Not GLP	Swiss Mouse 5/sex/group	0, 100/50, 62.5, 75 i.v. (bolus)	62.5 (M) 75 (F)	75 (M) > 75 (F)	**100 mg/kg**: death of the only M dosed, ataxia, convulsion **≥ 50 mg/kg**: ↓ body weight ↓ WBC, neutrophils, lymphocytes, monocytes, eosinophils, RBC, hemoglobin, hematocrit, reticulocytes, thrombocytes, ↑ ALP Thymic depletion Single cell necrosis of small intestinal epithelium **≥ 62.5 mg/kg**: Ataxia, convulsion ↓ triglycerides and calcium (F) Bone marrow depletion **75 mg/kg**: Mortality (1M) ↓ general activity Atrophy/depletion of Peyer's patches
Momparler, Ref. 46 Not GLP	CD2F1 Mouse 10M/group	25.6, 46.4, 73.7 8-h i.v infusion.	< 25.6	25.6	**≥ 25.6 mg/kg**: mortality (1/10 at LD, 4/10 at MD and 7/10 at HD) **≥ 46.4 mg/kg**: ↓ body weight

Momparler, Ref. 46 Not GLP	CD2F1 Mouse 4 – 15 M/group 5 – 20 F/group	M : 0, 11.1, 22.2, 29.5, 33.3, 44.4 F : 0, 11, 18, 22, 33, 44 12-h i.v. infusion	22.2 (M) 11 (F)	29.5 (M) 18 (F)	Males: **≥ 22.2 mg/kg**: ↓ body weight **≥ 29.5 mg/kg**: Mortality (6/15 at 29.5, 4/5 at 33.3 and 5/5 at 44.4 mg/kg) Females: **≥ 18 mg/kg**: ↓ body weight Mortality (7/20 at 18, 3/5 at 22, 3/5 at 33 and 5/5 at 44 mg/kg)
Momparler, Ref. 46 Not GLP	CD2F1 Mouse	M : 29.2 F : 18.0 12-h i.v. infusion	29.2 (M) 18.0 (F)	> 29.2 (M) > 18.0 (F)	D7: Bone marrow hypoplasia, atrophy of thymus, necrosis of small intestine D28: testicular atrophy (reversible)
Momparler, Ref. 46 Not GLP	Beagle Dog 1 or 2/sex/group	3, 5, 7 12-h i.v. infusion	3	5	**3 mg/kg**: soft, bloody stools, loss of appetite, inactivity, vomiting Body weight loss (reversible) Leukopenia and thrombocytopenia ↓ red blood cells, hematocrit, hemoglobin Narrow and shrunken seminiferous tubules (some evidence of spermatogenesis) Accumulation of hyaline material in kidney tubules **5 mg/kg**: Mortality (1M/1) related to bone marrow hypoplasia and ulceration of the GI tract Leukopenia, thrombocytopenia and erythropenia **7 mg/kg**: Mortality (1F/2) ↓ general activity, fever, salivation, watery or mucoid stools, slight cough ↑ PAL and cholesterol Leukopenia, granulocytopenia, thrombocytopenia and erythropenia Atrophy of lymphoid tissue Epithelial damage of intestine ↓ nucleated cells and absence of megakaryocytes in bone marrow Congestion of liver Vacuolar degeneration of renal tubules
TOX8712 Not GLP	Cynomolgus Monkey 1/sex/group No histopathological examination	3.27, 6.55, 13.1, 26.2, 52.4 1-h i.v. infusion	52.4	> 52.4	**≥ 3.27 mg/kg**: ↑ reticulocytes counts (related to repeated blood sampling) ↓ leucocyte, neutrophil, eosinophil and monocyte count

In mice, mortality was observed at lower doses after i.v. infusion (around 20 mg/kg) compared to i.v. bolus (75 mg/kg). Deaths occurred at 5 mg/kg in dogs whereas no mortality was observed in monkeys up to 52.4 mg/kg.

The main effects were observed on the hematopoietic system (bone marrow hypoplasia and thymus atrophy in mice, leukopenia, granulocytopenia, thrombocytopenia and erythropenia, atrophy of lymphoid tissue and hypocellularity of bone marrow in dogs, decreased leucocytes count in monkeys).

Effects on testis was evident in mice (testicular atrophy at 29.2 mg/kg) and in dogs (seminiferous tubule atrophy at 3 mg/kg). The intestine was affected by the treatment (necrosis in mice, epithelial damage in dogs).

Repeat dose toxicity

Repeat-dose toxicity studies were conducted in mice, rats, dogs and rabbits (see Table below).

Table 6: Summary of repeat-dose toxicity studies

Study reference/ GLP compliance	Species/ Number/Sex/ Group	Dose (mg/kg/day) Route	Duration	NOAEL (mg/kg/ day)	Major findings
086647 GLP	Swiss Mouse 5M/group	0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 5.0 i.v. (bolus)	Once daily, 5 days a week for 2 weeks (*treatment prolonged for one week for animals receiving 0,1 and surviving animals receiving 0,25 and 0,5*)	0.1	**≥ 0.25 mg/kg**: Mortality (1/5 at 0.25, 3/5 at 0.5, 2/5 at 1 and 5/5 at 5 mg/kg) Lethargy, rough coat, swelling of the snout, pale skin ↓ body weight, ↓ food consumption Enlarged spleen urinary bladder and kidney. **≥ 0.5 mg/kg**: Small thymus, gray/white focus on the liver, pale liver **1 mg/kg**: Red testis, thickening of the cheek region and parotid glands **5 mg/kg**: Hunched posture, ptosis
079403 GLP	Swiss Mouse 20 and 30M/group	0; 0.25 i.v. (bolus)	Once daily, 5 days a week for 4 weeks + 4 weeks of recovery	< 0.25	↓ food consumption Rough coat Marked depletion of RBC (not reversible) and WBC ↓ Hb, Hct, MCHC, ↑ MCV, ↑ reticulocytes and lymphocytes, ↓ neutrophils ↓ thymus size, lymphoid atrophy, lymphodepletion ↓ testes weight, degeneration of testicle tubular epithelium, damaged seminiferous epithelium, necrotic germinal cells, ↓ number of spermatids and spermatozoas (testicular degeneration not reversible) ↑ spleen weight, ↑ spleen haematopoiesis ↓ myeloid:erythroid ratio, ↑ myeloid left shift index
FEAW-115 GLP	SD Rat 5 M/group	0, 1.2, 2.1, 3.6 3-h i.v. infusion	3 times a day, for 3 days	< 1.2	**≥ 1.2 mg/kg**: ↓ thymus and spleen weight **≥ 2.1 mg/kg**: ↓ WBC, ↓ absolute polymorphonuclear neutrophil count, ↑ glucose **3.6 mg/kg**: ↑ epididymis, kidney, prostate and salivary gland weight

FEAW-116 GLP	SD Rat 5/sex/group (TK: 6/sex/group)	0, 1.2, 2.4, 3.6 3-h i.v. infusion	3 cycles of treatment: 3 times a day, for 3 days + 25-day recovery period between dose-cycle	< 1.2	**≥ 1.2 mg/kg**: ↓ platelets, ↓ reticulocytes and eosinophil counts ↓ potassium (M) Testes tubular vacuolation, spermatid giant cells, ↓ number of spermatozoa **≥ 2.4 mg/kg**: Mortality (1F at MD and 1F at HD) Anemia (↓ RBC, Hb, Hct) normalized after each treatment cycle ↓ potassium (F) **3.6 mg/kg**: ↓ testes weight, ↓ epididymis weight (ns) testes germ cell depletion
FEAW-117 GLP	Beagle Dog 3/sex/group	0, 1.2, 2.4, 3.6 3-h i.v. infusion	3 cycles of treatment: 3 times a day, for 3 days + 25-day recovery period between dose-cycle[a]	< 1.2	**≥ 1.2 mg/kg**: Mortality (2M/3 and 2F/3 at LD, 3M/3 and 3F/3 at MD and 2M/3 and 3F/3 at HD) due to bone marrow depletion and enteropathy leading to bacterial proliferation. ↓ body weight and food consumption Soft, mucoid, bloody feces, vomiting, lethagy ↓ WBC, ↓ absolute polymorphonuclear neutrophil and lymphocytes counts, ↓ platelets ↓ monocytes, eosinophils, basophils and reticulocytes Mucosal and/or serosal congestion, mucosal hemorrhage and mucosal necrosis of small and large intestine and stomach Possible treatment-related alterations in the testes Hemorrhage in the lungs, thymus and adrenal cortex Lymphoid depletion in spleen, thymus, visceral and peripheral lymph node and gut-associated tissues **≥ 2.4 mg/kg**: ↑ glucose (M), ↓ sodium, chloride and calcium
FEAW-0125 Not GLP	NZW Rabbit 2 M/group	0, 2.4, 3.6, 4.8 3-h i.v. infusion	3 times a day, for 3 days	< 4.8	**≥ 2.4 mg/kg**: Myelosuppression, ↓ neutrophils, basophils, monocytes, platelets and reticulocytes **4.8 mg/kg**: Mortality (1M/2) Inappetence, thinning fur, “warm to touch” ↓ WBC and lymphocytes

FEAW-0126 GLP	NZW Rabbit 4/sex/group	0, 0.75, 1.5, 3.0[b] 3-h i.v. infusion	3 cycles of treatment: 3 times a day, for 3 days + 6-week recovery period between dose-cycle	< 0.75	**≥ 0.75 mg/kg**: Mortality (1M/4 at LD, 3M/4 and 1F/4 at MD and all animals at HD) associated with bone marrow hypocellularity, leucopenia, anemia, thrombocytopenia and immunosuppression-related pneumonia Scant, soft feces, inappetence Anemia, thrombocytopenia, leucopenia (reversible), ↓ testis weight, testicular atrophy **1.5 mg/kg**: ↓ body weight, ↓ phosphorus **3.0 mg/kg**: Enteropathy of the large intestine

ns: non significant
[a] Due to early deaths, the study was terminated during the first recovery period
[b] Given the deaths, which were attributable to bacterial or fungal infection at 0,75 and 1,5 mg/kg, in order to prevent further mortalities due to opportunistic infection, all animals were administered 5 mg/kg of Baytril (enrofloxin) for seven days beginning on the 6 day following dosing cycles 2 through 4. No additional deaths were observed after institution of this treatment.

Genotoxicity

Two formal *in vitro* genotoxicity studies were submitted.

Table 7: Summary of genotoxicity studies

Type of test / study ID / GLP	**Test system**	**Concentrations / Concentration range / Metabolising system**	**Results**
Ames test 065115 GLP	S. typhi TA1537, TA1538, TA98, TA1535, TA100	3.3 – 333 µg/plate without S9-mix 33.3 – 2000 µg/plate with S9-mix	Negative Microcolonies were observed at 100 µg/plate and higher
Gene mutation test 121848 Not GLP	L5178Y Mouse Lymphoma Cells	10 – 5000 µg/mL +/- S9-mix	Positive

The mutagenic potential of decitabine was also investigated in several literature studies.

In a study testing the induction of 6-thioguanine resistance in Chinese hamster ovary cells, decitabine was not mutagenic (Momparler, 1984c). It was also negative in an assay for mutation to ouabain resistance in CH3/10T1/2 mouse fibroblast or V79 Chinese hamster cells (Landolph, 1982) and in an assay for mutation to 6-thioguanine- or ouabain-resistant mutations in the baby hamster kidney cell line BHK-21/cl13 (Bouck, 1984).

However, decitabine induced chromosomal aberrations in FLEB14 cells (human pro-B-cell line) (Ji, 1997). It induced homologous mitotic recombination and minor mutagenicity in the wing somatic and recombinant test (SMART) in *Drosophila melanogaster*. Decitabine also produced chromosome anomalies in roots of *Vicia faba* (Fucik, 1970).

A published study reported on the mutagenic potential of decitabine in transgenic mice carrying an *E. coli* trans-reporter gene. The lac-I gene contains 94 CpG dinucleotides and a total of 299 cytosine residues in 1083 base pairs of coding sequence that could serve as targets for decitabine mutagenesis. Decitabine was randomly incorporated at both CpG and non-CpG cytosine residues (almost exclusively

at C:G base pairs). Various mutations following treatment with decitabine were observed. Of significance, 73% of the mutations induced by decitabine occurred at CpG sites implicating demethylation as a factor in mutagenesis (Jackson-Grusby, 1997).

Carcinogenicity

No conventional carcinogenicity studies were conducted. Data from literature are available (see Table below).

Table 8: Summary of carcinogenicity studies from literature

Reference	Species/ Number/ group	Dose (mg/kg/day) Route	Duration	NOAEL (mg/kg/ day)	Major findings
Thomas, 1992	Mouse C3H x GPDX 9 – 11 F/group	3 single dose of 2.5 mg/kg i.p.	Administration 5, 10 and 15 days after the start of goitrogen treatment	NA	Increased frequency of tumors in animals pretreated with goitrogen. No promotion of thyroid tumorigenesis without the presence of goitrogen-induced hyperplasia.
Laird, 1995	Progeny of mousea female 129/SV DnmtS/+ X Male C57BL/6 ApcMin/+	5µg/5 g body weight s.c.	Weekly for 14 weeks starting at 1 week of age	NA	Reduction of the number of polyps in Dnmt wild-type mice and in heterozygous mice for Dnmt.
Lanty, 1999	Mouse 3H/HeJ x A/J F1 hybrid	1.0/0.5b i.p. Tumor induction by NNK	Three times weekly for 24 weeks	NA	23% reduction tumor incidence and 42% reduction in tumor multiplicity. =>Prevention of tumor formation in a primary mouse lung tumor model.
Carr, 1988	Rat/F344 10 M/treated group	Decitabine: 2.5 THU: 27.5 i.p.	Three times weekly for 12 months	NA	No animal with tumors. All animal had testicular atrophy (reduced size of seminiferous tubules, absence of spermatogenesis, edema and focal interstitial fibrosis) In 2 animals: Leydig cell hyperplasia. => Non-carcinogenic in the Fischer rat after 12 months
Berger, 1997	Rat/SD 50/sex/group	0, 0.06, 0.3, 6.0 i.p.	5 times weekly for 86 weeks for LD and MD 3 injections every 12 weeks for 86 weeks for HD	0.06	Reduced survival time at 0.3 and 6 mg/kg Incidence of malignant tumors: Control: 40% in M and 30% in F 0.06 mg/kg: 36% in M and 44% in F 0.3 mg/kg: 68% in M and 74% in F 6.0 mg/kg: 94% in M and 74% in F Target organs: hematopoietic system, skeleton, nervous tissue, skin, mammary gland => Carcinogenic in SD rats after 86 weeks

NA: Not applicable

[a] Min mice develop multiple intestinal adenomas within the first few months of life.

Mice carrying the Min allele of the Apc gene (ApcMin/+) develop 100 or more intestinal polyps in the first 6 months of life, providing a model system for early stages of human colorectal cancer.

Dmnt mice carry germline mutations in the DNA methyltransferase gene (DnmtS/+). Heterozygous mice have approximately half wild-type levels of DNA methyltransferase expression, which is sufficient to maintain normal levels of DNA methylation. They appear phenotypically indistinguishable from their wild-type littermates.
[b] Mice were treated with 1 mg/kg for the first 12 weeks. Due to toxicity, the dose was reduced to 0.5 mg/kg for the following 12 weeks.

Reproduction Toxicity

No conventional reproductive and developmental toxicity studies were conducted. Data from literature are available (see Table below).

Table 9: Summary of reproductive and developmental studies

Reference	Species; Number/ sex/group	Dose (mg/ kg/day) Route	Study design	NOAEL (mg/kg /day)	Major findings
FERTILITY AND EARLY EMBRYONIC DEVELOPMENT					
Raman, 1995	Mouse/Albino Males	0, 1 i.p.	3 injections at 8-hour interval to 5-day old mice having only spermatogonial premeiotic cells	NA	Loss of methylation after treatment. Complete inhibition of differentiation from the gonial into spermatocytic stage. Testis devoided of meiocytes. Alteration of the expression of several polypeptides. =>Decitabine prevents DNA methylation, a key process in gene regulation for the proliferation and differentiation of gonals into primary spermatocytes.
Kelly, 2003	Mouse Males Dnmt1+/+ Females CD1	0, 0.05, 0.1, 0.15 i.p.	Males treated 3 times a week for 7 weeks and then mating with females during 6 days	0.05	No effect on survival, body weight gain, haematological parameters or mating behaviour **≥ 0.1 mg/kg**: ↓ testis weight, Histological abnormalities (disordered germ cell association, vacuolization, multinucleated cells) ↓ sperm number ↓ pregnancy rate ↑ pre-implantation loss These effects were dose-related
Oakes, 2007	Mouse Males Dnmt1+/+ and Dnmt1c/+ *	0, 0.1 i.p.	Males treated 3 times a week for 7 weeks and then mating with female	NA	↓ testis weight ↑ proportion of immotile spermatozoa ↓ motility characterics of motile spermatozoas ↓ fertilization ability ↓ survival of embryos to blastocyst stage Heterozygous mice were partially protected against the adverse effects of decitabine. => Decitabine specifically inhibits de novo methylation activity in male germ cells
EMBRYO-FETAL DEVELOPMENT					
Branch, 1996	Timed-pregnant CD1 Mouse	0, 0.3, 1.0 i.p.	Single injection on GD8, 9, 10 or 11 Sacrifice at GD17	< 0.3	**F0 Dams** No maternal effect **F1 Fetuses** **≥ 0.3 mg/kg**: ↓ fetal weight Supernumerary ribs Cleft palate

					1 mg/kg: ↓ survival (in GD9 treatment group only) Fused and missing ribs, fused vertebrae Vetebral defects (offset, irregularly shaped, fused and missing vertebrae) Hindlimb defects (phocomelia, meromelia, absent or reduced femurs, reduced fibulae and pelvic bones and reduction and curvature of the tibias) Tail defects (offset, fused, abnormally shaped) Digital defects (brachydactyly, oligodactyly, polydactyly and syndactyly) **F1 Pups** Locomotion abnormalities associated with appendicular defects
Branch, 1999	Timed-pregnant SD Rat	0, 0.4, 0.6, 1 i.p.	Single injection on GD 9, 10, 11 or 12 Sacrifice at GD20	< 0.4	**F0 Dams** No maternal effect **F1 Fetuses** **≥ 0.4 mg/kg**: Complete resorption (GD9 treatment group) Vertebral defects, fused and supernumerary ribs **≥ 0.6 mg/kg:** ↓ number of live fetuses Foredigits (syndactily and oligodactility) and hindigits defects **1 mg/kg:** Missing ribs Coccygeal defects (offset and unossified) Cleft palate Exencephaly Exophtalmia Hindlim defects (meromelia and tarsal fusion) Long bone defects (reduction in size and reduced ossification)
POSTNATAL DEVELOPMENT					
Cisneros, 2003b	Timed-pregnant CD-1 mice	0, 1 i.p.	Single injection at GD10 F1 animals were mated with control animals	NA	**F0 Dams** No maternal effect **F1 animals** ↓ Body weight Sypernumerary ribs, cleft palate, tail and hindlimbs defects ↓ Testis and epidydimis weight ↓ Reproductive parameters ↓ Spermatid heads per testis ↓ Pregnancy rate Males more affected than females
Cisneros, 2003a	Timed-pregnant CD-1 mice	0, 1 i.p.	Single injection at GD10 F1 animals were mated with control animals	NA	**F0 Dams** No maternal effect **F1 animals** ↓ Body weight Teratogenic effects ↓ mating capacity and fertility (more

					pronounced in males than in females) No effect on cortisterone or glucose levels ↓ IGF-1 levels in males
Cisneros, 2004	Timed-pregnant CD-1 mice	0, 1 i.p.	Single injection at GD10 F1 animals were mated with control animals	NA	**F0 Dams** No maternal effect **F1 Fetuses** Teratogenic effects ↑ Ratio of males to females **F1 Male Pups** ↓ mating behaviour (↓ vaginal plug, mount latency and number of mounts) and ↓ sexual interest Normal presence of Sry gene

* Mice Dnmt1c/+ are heterozygous for nonfunctional Dmnt1 gene

Table 10: Summary of juvenile studies

Report No GLP compliance	Species/ Number/ group	Dose (mg/kg/day) Route Duration	NOAEL (mg/kg/day)	Major findings
TOX9539 Not GLP	SD Rat 8/sex/group	<u>Cohort 1</u> 0, 0.2 s.c. (PND7-11; 14-18) + 0.1 i.v. (PND23-23; 31-34) 0.7 s.c. (PND7-11; 14-18) + 0.3 i.v. (PND23-23; 31-34)) 2.0 s.c. (PND7-11; 14-18) + 1.0 i.v. (PND23-23; 31-34) <u>Cohort 2</u> 0, 0.2 s.c. (PND12-16) + 0.1 i.v. (PND21-24; 29-32) 0.7 s.c. (PND12-16) + 0.3 i.v. (PND21-24; 29-32) 2.0 s.c. (PND12-16) + 1.0 i.v. (PND21-24; 29-32) 5-day recovery period	< 0.2/0.1 mg/kg	<u>Cohort 1</u> **0.2/0.1 mg/kg**: ↓ Body weight (reversible) No effect on timing of vaginal opening Anisocytosis, macrocytosis, ↑ reticulocytes, ↓ WBC and platelet **0.7/0.3 mg/kg**: Pallor, ↓ Body weight (reversible in F) No effect on timing of vaginal opening ↓ Hct, Hb, RBC (reversible) Anisocytosis, macrocytosis,↑ reticulocytes, ↓ WBC and platelet Small reproductive organs in 4 M of the same litter (considered litter-related) **2.0/1.0 mg/kg**: Treatment suspended on PND18 Premature sacrifice on PND19/20 ↓ body weight and weight loss Deterioration of clinical condition ↓ RBC, reticulocytes, lymphocytes and platelets, anisocytosis, macrocytosis Pallor of extremities, pale liver, kidney, spleen; pale, thin, watery blood <u>Cohort 2</u> **0.2/0.1 mg/kg:** No effect on timing of vaginal opening ↓ WBC and platelet **0.7/0.3 mg/kg:** No effect on timing of vaginal opening Anisocytosis, macrocytosis,↑ reticulocytes, ↓ WBC (after recovery) **2.0/1.0 mg/kg:** ↓ body weight and weight loss Pallor, piloerection, dull eyes ↓ body temperature Premature sacrifice of animals from litter 20 (PND27) In remaining animals: recovery of RBC and lymphocyte concentrations with ↑ reticulocytes, prothrombin and partial thromboplastin time similar to controls.

TOX9540 GLP	SD Rat 30/sex/group	<u>Cohort 1</u> 0, 0.3 s.c. (PND7-10; 15-17) + 0.1 i.v. (PND21-24; 29-31 and 35) 0.9 s.c. (PND7-10; 15-17) + 0.3 i.v. (PND21-24; 29-31 and 35) <u>Cohort 2</u> 0, 0.3 s.c. (PND11-14; 18-20) + 0.1 i.v. (PND25-28; 32-35) 0.9 s.c. (PND11-14; 18-20) + 0.3 i.v. (PND25-28; 32-35) 35-day recovery period	< 0.3/0.1	**≥ 0.3/0.1 mg/kg:** Small testes ↓ Body weight gain, ↓ ulna growth ↓ WBC (not reversible) Small thymus, epididymides, seminal vesicles and prostate and testes, ↓ testis weight (not reversible) ↓ sperm count (not reversible), spem concentration Lymphoid depletion Changes in the bone marrow (reversible) **0.9/0.3 mg/kg:** Pallor Delayed sexual maturation ↓ RBC and RBC parameters (partially reversible) ↑ sperm abnormalities ↑ pre-implantation loss Seminiferous tubular atrophy/degeneration (not reversible) Oestrous cycle length, pre-coital interval, mating performance and fertility not affected when treated males were paired with untreated females and vice versa.

Toxicokinetic data

Table 11: Exposure margin based on AUC and Cmax

Type of study	Species	Duration	NOAEL (mg/kg/day)	AUC decitabine (ng.h/mL) at NOAEL[a]	Cmax decitabine (µg/mL) at NOAEL[a]	Exposure margin[b] based on	
						AUC	Cmax
Single dose	Monkey	Single dose	< 3.27	-	90.65	-	< 0.8
Repeated dose	Rat	3 treatment cycles	< 1.2	-	228	-	< 2.1
	Rabbit	3 treatment cycles	< 0.75	254c	83.5c	< 0.4	< 0.8
	Dog	1 treatment cycle	< 1.2	-	154	-	< 1.5
Juvenile toxicity	Rat	4 treatment cycle	< 0.3 s.c / 0.1 i.v.	668	-	< 1.1	-

a: Gender average values
b Animal/human exposure ratios calculated from decitabine human predicted values of Cmax= 107 ng/mL and AUC = 580 ng.h/mL in a typical patient receiving daily 1-hour infusion of Dacogen 20 mg/m²/day (= 0.6 mg/kg/d for a 60-kg and 1.8-m² person) over 5 days every 4 weeks (Population PK analysis)
c Data obtained after a single intravenous infusion of decitabine at 0.25 mg/kg (FK6682)

Local Tolerance

No specific studies were conducted.

However, decitabine has been evaluated extensively in animals and humans using the intended, intravenous route of administration, particularly following multiple cycles of repeated i.v. infusion. In animals species, the multi-cycle i.v. infusion toxicity studies in rabbits (FEAW-0126) and dogs (FEAW-117) included histopathological evaluation of the site of administration (femoral vein) and demonstrated only minimal pathological changes, indicating good local tolerance to decitabine. Up to the highest concentration tested in the monkey no clear decitabine-related changes were visible at the site of venous catheter implantation (AA71776).

Other toxicity studies

Two GLP-compliant studies on impurities were submitted (see Table below).

Table 12: Summary of studies on impurities

Study reference / GLP compliance	Species / Number / Sex / Group	Test substance	Dose (mg/kg/day) Route	Duration	NOAEL (mg/kg/day)	Major findings
079831 GLP	Swiss Mouse 10/sex/group	Degraded decitabine solution (left 24h at room temperature after reconstitution)	85, 115, 150, 200, 250 i.v. (bolus)	Single injection	115 DL50 = 215 (M) 237 (F)	**150 mg/kg**: Mortality (1M/10) Hunched posture (M), Piloerection **200 mg/kg**: Mortality (4M/10 and 2F/10), lethargy (F), piloerection **250 mg/kg**: Mortality (7M/10 and 6F/10), lethargy
TOX8789 GLP	NZW Rabbit 5/sex/group (3/sex/gp sacrificed 4 days after last infusion and 2/sex/gp* sacrificed 43 days after last infusion	Decitabine Decitabine spiked with impurities Composition of the solution before administration: RS11: 1.79% RS03: 2.37% RS04: 3.99% RS05: 1.97% α-anomer:0.05% RS06/07:0.03 % RS08: 4.45% RS09:0.04% RS10: 1.00%	0 1.5 1.5 3-h i.v. infusion	3-h infusion , 3 times a day for 3 days	NA	In both treated groups: Mortality (1M+1F with decitabine and 2M with decitabine+impurities) Comparable clinical signs Body weight loss, ↓ food consumption ↓ RBC parameters, ↓ reticulocytes, ↓ platelets, ↓ WBC (especially neutrophils) (reversible) ↑ glucose and urea ↓ thymus weight ↓ testes weight, ↓spermatogenesis, ↑ spermatid giant cells and ↑tubular dilation Hypospermia in epididymis ↓ paracortical and germinal center development in mesenteric lymph nodes ↓ cellularity in thymus ↓ hematopoietic cells in bone marrow (M) Intestinal villous atrophy

*To prevent opportunistic infections during the study, recovery animals (including the control animals) were treated with enrofloxacine (Baytril®, 5 mg/kg by intramuscular administration) twice a day, for 7 days, starting on Day 6.

2.3.5. Ecotoxicity / environmental risk assessment

Table 13: Summary of main study results

Substance (INN / Invented Name): Decitabine			
CAS-number (if available): 2353-33-5			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	Not specified	-0.32	No Potential PBT

Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
$PEC_{surfacewater}$, default or refined (e.g. prevalence, literature)	0.000058	µg/L	< 0.01 threshold

2.3.6. Discussion on non-clinical aspects

The data about primary and secondary pharmacodynamics are based on published literature.

Decitabine is a DNA methyltransferase inhibitor. When specifically incorporated into DNA, it covalently links with DNMT. The enzyme is therefore trapped and not available for further methylation. This induces the hypomethylation of several gene promoters and allows re-expression of silenced genes such as tumor suppressor genes that can prevent the transformation to malignant cells and induce cell differentiation (p15/INK4b, p16, p14, SHP-1, p21WAF1, p73, C/EBPδ).

Decitabine can also induce re-expression of gene involved in immunogenicity and immune recognition of cancer cells. It could enhance the expression of tumor-associated antigens and the recognition of tumor cell by cytotoxic T cells.

The depletion of DNMT could not only be due to covalent link with DNA. It has been demonstrated decitabine may also induce the ubiquitination and proteasomal degradation of DNMT1.

Decitabine also acts by inducing cytotoxicity. Incorporation of decitabine into DNA generates DNA-DNMT adducts that interfere with DNA synthesis during cell replication and produce apoptosis. The induction of apoptosis appeared related to the re-expression of the tumor suppressor p73.

Additionally, decitabine has an effect on angiogenesis; it decreased vessel formation in different tumor models.

In vivo, decitabine showed a strong activity in murine leukemia models (AKR, P388 and L1210). It increased the survival of treated animals. This activity was dose-dependant up to a certain dose after which a plateau is reached or survival even decreases. The same relationship was observed in a rat myeloid leukemia model.

The effects of the combination of decitabine with other anticancer drugs were studied. These studies showed the importance of sequencing when considering combination treatment with decitabine.

Several potential mechanisms of resistance to decitabine have been investigated *in vitro* (decrease of deoxycytidine kinase activity, increase of dCTP pool, increase of cytidine deaminase activity, low levels of decitabine transporter hENT) but no cleat-cut resistance pattern has been established in clinical studies.

Regarding secondary pharmacodynamics, decitabine showed activity in sickle cell disease treatment by allowing re-expression of HbF gene. It was able to induce cell growth without and differentiation in normal hematopoietic cells. *In vitro*, the α-anomer showed activity and less cytotoxicity than decitabine.

Regarding safety pharmacology, decitabine was tested in an Irwin's test in mice, an in vitro hERG study and in telemetered conscious cynolmogus monkeys. In the Irwin's test, there were no effects on rectal temperature, body weight and sensory-motor functions. At the highest dose (50 mg/kg), a non-statistically significant slight neuro-depression was observed until 5 hours post-dose. Regarding effects

on the cardiovascular and respiratory system, decitabine did not induce relevant effects on IKr current in hERG-transfected cells up to 30 µM. Non physiologically relevant increase of IKr current were observed at concentrations ($3x10^{-6}$ M and $3x10^{-5}$ M) higher than Cmax in humans. *In vivo*, the only observed effect was a decrease in heart rate within 4 hours post-dosing conscious telemetered monkeys treated with 52.4 mg/kg, a dose leading to an exposure far exceeding human exposure.

Pharmacokinetics of decitabine was studied in mice, rats, rabbits and monkeys. The profile was roughly the same in every species.

After a single administration of decitabine, the main toxic effects were observed on the hematopoietic system (bone marrow hypoplasia and thymus atrophy in mice, leukopenia, granulocytopenia, thrombocytopenia and erythropenia, atrophy of lymphoid tissue and hypocellularity of bone marrow in dogs, decreased leucocytes count in monkeys). In mice, mortality was observed around 20 mg/kg after i.v. infusion and at 75 mg/kg after i.v. bolus. Deaths occurred at 5 mg/kg in dogs whereas no mortality was observed in monkeys up to 52.4 mg/kg.

In repeat-dose toxicity studies, Decitabine was tested in mice up to once daily for 4 weeks, in rats and rabbits for 3 cycles of 3-time daily injections for 3 days and in dogs for 3 days of 3-time daily injections. In all tested species, the main toxicity was hematological with pronounced anemia, leucopenia and thrombocytopenia related to bone marrow alteration. These effects were often reversible. In most of studies, mortality occurred, even at the lowest doses. Deaths in dogs and rabbits were attributed to infection secondary to immunosuppression. The testes were a target organ in mice, rats, dogs and rabbits. Decitabine induced irreversible testicular toxicity with testicular atrophy, decreased germ cells and spermatozoa numbers. The intestine was also damaged in dogs (congestion and necrosis) and rabbits (enteropathy).

The NOAEL in mice was 0.1 mg/kg. No NOAEL can be established in the other species; they were < 1.2 mg/kg in rats and dogs and < 0.75 mg/kg in rabbits.

The genotoxicity assessment of decitabine was based on two conventional *in vitro* tests and non-conventional published studies. In the Ames test, decitabine was not mutagenic. However, it should be noted that all the recommended strains were not used (*E. coli* or *S. typhi* TA102 were missing) and bacteriotoxicity was observed at low concentrations. It was clearly mutagenic in a gene mutation test in mouse lymphoma cells. Published studies gave conflicting results with regards to genotoxicity. However, decitabine induced chromosomal aberrations in human pro-B cells and was positive in the SMART test in Drosophila melanogaster. Furthermore, it was positive *in vivo* in transgenic mice. Based on the submitted data, decitabine should be considered as genotoxic.

No conventional GLP-compliant carcinogenicity studies were submitted. Studies using different protocol are available in the literature and gave conflicting results. However, in the study using a protocol close to the conventional 2-year study, decitabine showed a clear carcinogenic potential. Therefore, based on the submitted data, decitabine should be considered as a potential carcinogen.

Fertility, embryofetal development and postnatal development studies are published studies.

Decitabine affected male mice fertility. Decreased testis weight associated with histological abnormalities and decreased fertility parameters (sperm number, spermatozoa motility, early embryos survival, pregnancy rate, pre-implantation loss) were observed at 0.1 mg/kg and higher. The alteration of fertility can be explained by the fact that decitabine inhibits *de novo* methylation, a key process in gene regulation for development of germinal cells. No human data on the effect of decitabine on fertility are available. Because of the possibility of infertility as a consequence of Dacogen therapy, patients should seek advice prior to initiation of treatment with Dacogen.

Decitabine was clearly teratogenic in mice and rats. It induced a wide-range of skeletal malformations at doses of 0.3 mg/kg in mice and 0.4 mg/kg in rats while no maternal toxicity was observed. There are no adequate data on the use of Dacogen in pregnant women. The potential risk for humans is unknown. Based on results from animal studies and its mechanism of action, Dacogen should not be used during pregnancy and in women of childbearing potential not using effective contraception. If Dacogen is used during pregnancy, or if a patient becomes pregnant while receiving this medicinal product, the patient should be apprised of the potential hazard to the foetus.

Decitabine-induced changes in gene expression may play a role in the abnormal embryonic development.

Decitabine reduced mating capacity and behaviour and decreased fertility in mice exposed *in utero*. The adverse effects were more pronounced in males than in females. In F1 males, decreased IGF-1 levels and altered sexual behaviour were also observed.

The effects observed in juvenile animals were the same as in adult animals. The hematopoietic system was the main target organ and irreversible testicular toxicity was also caused by the treatment in immature males.

No safety margins can be calculated for repeat dose in mice, carcinogenicity or reproductive toxicity because some of the submitted studies were from literature and no toxicokinetic measurements were included in the protocols.

NOAEL could not be defined in repeat dose toxicity studies due to hematological toxicity and/or mortality. Therefore, safety margins were often around 1.

Decitabine was administered via the intended clinical route (i.e. intravenous) in toxicity studies. No concern about local tolerance was raised from these studies or from clinical practice.

In mice, after a single injection, degraded decitabine showed similar toxicity to non-degraded decitabine as reported in studies 079392 and 079379. However, the relevance of this study is limited since no analysis of the degraded solution is performed. In a 3-day study, rabbits were treated with decitabine or decitabine spiked with impurities. No obvious differences were noted between the treated groups.

Decitabine PEC surfacewater value is below the action limit of 0.01 µg/L and is not a PBT substance as log Pow does not exceed 4.5.

Therefore decitabine is not expected to pose a risk to the environment.

2.3.7. Conclusion on the non-clinical aspects

The main toxic effects were myelosuppression, inhibition of spermatogenesis and intestinal enteropathy. All toxicities were reversible with exception of the testicular toxicity, which did not fully reverse after a recovery period of up to 6 weeks in rabbits. Most of the toxicities observed from treatment with decitabine are likely to be related to the cytotoxicity of the molecule.

The submitted non clinical data support the intended indication.

Decitabine was highly toxic in animals where it induced death and severe haematological and testicular toxicity. It is also considered genotoxic, carcinogenic, teratogenic and it impairs fertility.

2.4. Clinical aspects

2.4.1. Introduction

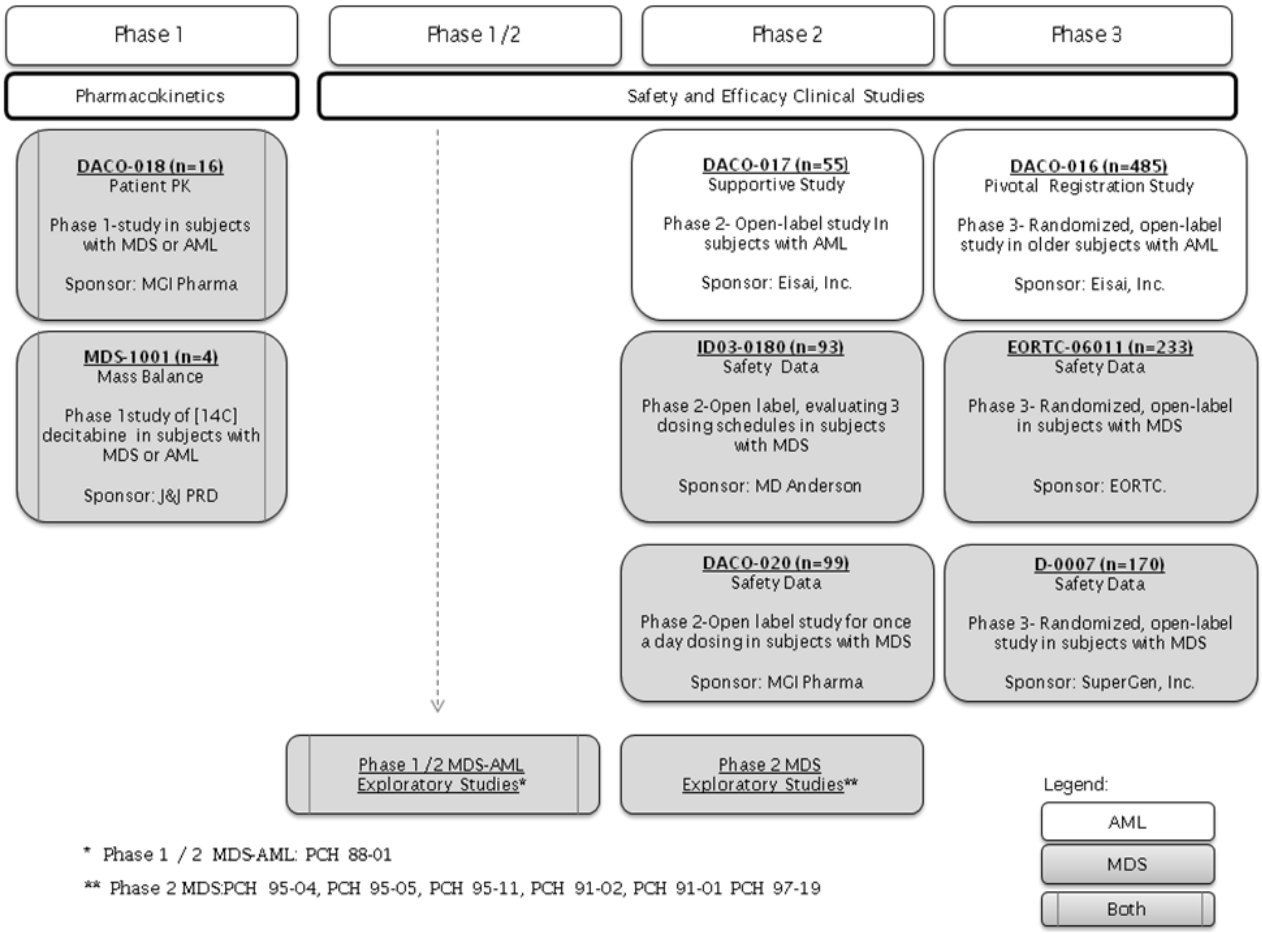
GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant. The inspections of 3 investigator sites were initiated within the scope of the centralised application procedure for DACOGEN. The overall evaluation of the findings observed at the inspected investigational sites shows that the investigational sites are overall GCP compliant and there is nothing identified to suggest that the data collected at these sites are unreliable.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Figure 1:



2.4.2. Pharmacokinetics

An overview of all clinical studies that contributed to the PK characterisation of Dacogen for the proposed indication is shown in the table below and all the studies included patients diagnosed with AML or myelodysplasia (MDS). No data are available in healthy subjects.

Table 14:Clinical Pharmacology Studies included in the PK Analysis (n= 63)

Study ID (No subjects)	Objectives	Patient Population	Dosage (IV)	PK sampling times	Included in Population PK (n= 59)
Phase I					
MDS-1001[a] (n=4)	PK	AML/MDS	20 mg (3 h infusion)	Up to 96 hours post dose	No
DACO-018[b] (n=14)	PK	AML/MDS	15 mg/m^2 tid (3 h infusion) Day 1-3 (6 week-cycle; 2 cycles)	Day 1-3 # 1: Predose, 1h, 2h, 2h55min, 3h 5min,3h 15min, 3h 30min, 4h, 4h 30 min, 5h, 7h, 8h; Day1-3 #2: Predose,2h 55 min	Yes
Phase II					
DACO-017 [b] (n=11)	CR/PK	AML	20 mg/m^2 od (1 h infusion) Days 1-5 (4 week- cycle)	Day 5 #1: Predose, 30, 60, 65, 75, and 90 min, 2h, 3h, 4h	Yes
DACO-020 [b] (n=11)	ORR/PK	MDS	20 mg/m^2 od (1 h infusion) Days 1-5 (4 week- cycle)	Day 5 # 1: Predose, 30, 60, 65,75, and 90 min, 2h, 3h, 4h	Yes
Phase III					
DACO-016 [b] (n=23)	OS/PK	AML	20 mg/m^2 od (1 h infusion) Days 1-5 (4 week- cycle)	Day 5 # 1: Predose, 60 and 90 min, 2h, 3h	Yes

[a] single dose mass-balance study
[b] multiple dose study
tid: 3 times a day (8 hourly)
od: once a day
#: cycle
CR= complete remission; ORR= overall response rate; OS= overall survival; PK= pharmacokinetics

A total of 59 patients (44 males and 15 females) contributed to the PK population analysis. The characteristics of this PK population were as follows:

- Median (range) age, weight, BSA and BMI were 70 years (40-87 years), 77 kg (58-129 kg), 1.9 m^2 (1.6-2.5 m^2) and 26.7 kg/m^2 (18.7-39.7 kg/m^2), respectively.
- Race was primarily white with only 2 black patients and 1 patient from other race group.
- Normalized creatinine clearance was within the normal range for 19 (32.2%) patients. Thirty three (55.9%) and 7 (11.9%) patients had respectively mild (50-80 mL/min/1.73m^2) and moderate (30-50 mL/min/1.73m^2) renal impairment.
- The patient population included 18 (30.5%) MDS patients and 41 (69.5%) AML patients.

Absorption

As Dacogen is administered by intravenous (IV) route it is 100% bioavailable. Therefore, no biopharmaceutical studies were conducted.

Bioequivalence studies were not required as the formulation used in the clinical studies is the same as the intended commercial formulation.

Distribution

After IV infusion of decitabine 20 mg/m^2 over 1 hour over 5 consecutive days the PK of decitabine follows a linear 2-compartment model with rapid elimination from the central compartment and slow distribution to the peripheral compartment with a large volume of distribution at steady state (Vd_{ss}) of 116 L. Steady-state concentrations are reached within 0.5 hour.

Decitabine concentrations decline biexponentially after the end of the infusion and are measurable up to 4 hours after the start of the infusion. 75% of the maximum concentration level is reached within 19 minutes. After the stop of infusion the concentration drops to 25% of the maximum level within 20 minutes.

In vitro data showed a negligible plasma protein binding (<1%). Additionally, decitabine is a poor P-gp substrate as well as a poor P-gp inhibitor, suggesting that there is limited potential for inhibition of P-gp in vivo.

Elimination

In the human mass balance study, the predominant route of excretion of total radioactivity was via the kidneys with approximately 90% excreted in the urine. Unchanged drug in urine accounted for approximately 4.2% of the total radioactive dose. The mean cumulative excretion of total radioactivity in feces accounted for approximately 0.48% of the dose. The high total body CL (mean = 267 L/h), low urinary excretion of unchanged drug in the urine (4.2% of the dose), and high urinary excretion of metabolites indicates that decitabine is predominately eliminated by metabolism. It is clear that decitabine CL is much higher than the normal hepatic blood flow (90 L/h) which is due to the metabolism of decitabine by cytidine deaminase which is present not only in the liver but in the kidneys, intestinal epithelium, and blood, as well.

Intracellularly, decitabine is activated through sequential phosphorylation via phosphokinase activities to the corresponding triphosphate, which is then incorporated by the DNA polymerase. In vitro experiments with microsomes suggested that there was only a minor involvement of cytochrome P450 (CYP) enzymes in the degradation of decitabine.

The results from the human radiolabeled mass-balance study indicated that the CYP system is not involved in the metabolism of decitabine.

Decitabine is primarily metabolized by enzymatic degradation. In vitro experiments with cytosolic fractions, and by making use of inhibitors of cytidine deaminase (ie, tetrahydrouridine [THU]), demonstrated that metabolism of decitabine is mainly mediated through liver, kidney, gastro-intestinal and/or blood cytidine deaminase rather than by CYP450 isoenzymes. Results from the mass-balance study showed that unchanged decitabine in plasma accounted for approximately 2.4% of total radioactivity in plasma. The unidentified metabolites M1 and M2, and M4 (the latter confirmed to be formed by oxidative deamination combined with ring opening and deformylation) accounted for approximately 48.2% to 57.0% (sum of M1 and M2) and 31.2 to 40.3% (M4) of the injected radioactivity in plasma. Although the structural identity of the metabolites M1 and M2 is not definitively known, evidence was collected that the pivotal toxicology species are also exposed to the 3 major human plasma metabolites. The major circulating metabolites do not contribute to the pharmacological activity, since the 4-amino-1,3,5-triazin-2(1H)-one ring is no longer intact in metabolite M4, and since the polar metabolites M1 and M2 have lost the sugar moiety and are likely to result from cytidine deaminase-mediated oxidative deamination.

No pharmacogenomics evaluation has been conducted.

Dose proportionality and time dependencies

No data have been provided on dose proportionality. A comparison of dosing regimens is presented in the table below.

Table 15- Comparison of dosing regimens PK parameters

Regimen	Total Dose (mg/m^2)	C_{max} (ng/mL)	AUC_{cum} (ng*hr/mL)	Time after start of infusion to $0.75*C_{max}$		Time after end of infusion to $0.25*C_{max}$	
				hr	min	hr	min
20 mg/m^2 1-hr infusion ever 24 hours for 5 days	100	107 (88.5-129)	580 (480-695)	0.31 (0.26-0.34)	19 (16-20)	0.33 (0.27-0.36)	20 (16-22)
15 mg/m^2 3-hr infusion every 8 hours for 3 days	135	42.3 (35.2-50.6)	1161 (972-1390)	0.14 (0.01-0.19)	8 (6-11)	0.14 (0.1-0.19)	8 (6-11)

PK parameters were independent of time with no dependency on study cycle and no accumulation observed within the cycle.

Special populations

According to the population PK, the inter-individual variability was 70.6% and 55.7% for central volume and clearance, respectively. Intra-individual variability of decitabine concentrations was moderate (CV=31.6%).

Impaired renal function: The PK of decitabine have not been formally studied in patients with renal insufficiency. Results from the mass balance study in cancer patients with radioactive 14C-decitabine showed that approximately 90% of the administered dose of decitabine is excreted in the urine with only 4.2% of the unchanged drug. The major circulating metabolites are believed to be not pharmacologically active as described above. In addition, population PK analysis indicated no

significant PK parameter dependencies on normalized creatinine clearance, an indicator of renal function. Thus, decitabine exposure is not likely to be affected in patients with impaired renal function.

Impaired hepatic function: The effects of hepatic impairment on the PK of decitabine have not been formally studied. Results from human mass-balance study and in vitro experiments mentioned above indicated that the CYP enzymes are unlikely to be involved in the metabolism of decitabine. It is unknown whether or not hepatic impairment may have an effect on the cytidine deaminase which is mainly responsible for decitabine metabolism, but it seems unlikely since this enzyme is not only found in the liver, but in the kidneys, intestinal epithelium, and blood as well. In addition, population PK analysis indicated no significant PK parameter dependencies on total bilirubin concentration despite a wide range of total bilirubin levels (0.2 – 2.9 mg/dL). Thus, decitabine exposure is not likely to be affected in patients with impaired hepatic function.

Gender : Population PK analysis of decitabine showed that men had a slightly higher CL (12.1%) than women. However, this difference is small relative to the inter-individual variability in CL (55.7%) and thus it is not clinically relevant.

Race: Most of the patients studied were Caucasian (95%). There seemed to be no apparent race effect on decitabine exposure from the limited data available.

Weight: The volume of distribution at steady state is proportional to body weight. Clearance has been shown to be proportional to BSA. Dosing by BSA would provide similar exposures (AUC) for patients of different body sizes.

Elderly: According to the population PK, Decitabine PK were not dependent on age (range studied 40 to 87 years; median 70 years).

Children: No PK data in children have been submitted.

Pharmacokinetic interaction studies

Decitabine metabolism is not mediated by the CYP450 enzyme system but by oxidative deamination. In vitro tests of decitabine inhibition and enzyme induction of various human CYP 450 enzymes showed decitabine does not inhibit nor induce CYP enzyme systems at concentrations more than 20 fold above the therapeutic plasma level. These tests included the following:

- Inhibitory potential of decitabine towards 6 major human CYP isoenzymes (CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) in pooled human liver microsomes
- Induction potential of decitabine towards 5 major human drug-metabolizing enzymes (CYP1A2, CYP2B6, CYP2C9, CYP3A4/5 and CYP2E1) using cultures of primary human hepatocytes

The bidirectional permeability of decitabine was studied in MDR1 transfected MDCK cell monolayers, both in absence and presence of cyclosporine A, a known P-gp inhibitor. It was demonstrated that decitabine was a poor P-gp substrate, suggesting that its biodistribution is not dependent on the expression or activity of this efflux transporter protein. Decitabine was also shown to be a poor P-gp inhibitor. As plasma protein binding of decitabine is negligible (<1%), interactions due to displacement of more highly protein bound drugs from plasma proteins are not expected.

No formal clinical drug-drug interaction studies have been conducted for decitabine.

2.4.3. Pharmacodynamics

Mechanism of action

DNA methylation represents one of the major epigenetic modifications of the genome involved with regulation of gene expression without affecting the primary DNA sequence. The methylation of cytosine residues at cytosine-guanosine dinucleotides (CpG islands) in DNA is mediated by DNA methyltransferases (DNMTs). DNA methylation is associated with the control of gene expression. Hypermethylation of CpG islands is seen in MDS and AML tumour types.

After cellular uptake and phosphorylation, decitabine is incorporated into DNA where it irreversibly inhibits DNMTs through covalent adduct formation with the enzyme. The trapping of DNMT results in reduced DNA methylation and re-expression of silenced genes that control tumour development and progression as well as cell differentiation.

In addition the incorporation of decitabine into DNA can lead to cytotoxicity via a mechanism that remains under investigation.

A further anti-tumour mechanism is through the stimulation of immune pathways by the re-expression of tumour antigens involved in cancer cell surveillance and elimination.

Primary and Secondary pharmacology

Primary pharmacology

- Antiproliferative effect

In several leukemic cell lines from murine and human origin and at concentrations that inhibited cellular DNA methylation and reduced DNMT activity in cell extracts, decitabine induced morphological and functional differentiation.

A dose- and time-dependent inhibition of proliferation was observed:

> Lower concentrations of decitabine seem to be optimal to inhibit DNA methyltransferase, induce DNA hypomethylation, whilst higher concentrations will increase the drug-induced cytotoxicity. In human leukemic cell lines, cellular differentiation and inhibition of DNA methylation reached a plateau, or even decreased above concentration of 0.5 to 1 µM.
>
> Incorporation into DNA requires transition of target tumor cells through the S-phase of the cell cycle. Decitabine does not induce cell cycle growth arrests that could be self-limiting to decitabine incorporation into DNA. Once incorporated into DNA, the trapping of DNMT and therapeutic effects of decitabine will persist in the absence of circulating plasma levels of decitabine. Therefore, intermittent schedules of decitabine administration should be effective in managing hematological malignancies given the persistence of the active DNA-decitabine complex.

Decitabine showed strong anti-tumour activity in several in vivo pre-clinical leukemia models and such activity was dose dependent in all studies and schedule dependent. Both extending duration of infusion or by repeating shorter courses of treatment over several days increased the antitumor effects.

- Decitabine resistance

Phosphorylation by deoxycytidine kinase represents the initial step to decitabine activation prior to incorporation into DNA. Complete loss or reduction of deoxycytidine kinase activity results in resistance to decitabine.

Secondary pharmacology

It has been demonstrated that decitabine enters the CNS but no relevant effects on CNS were seen in preclinical studies.

In addition, the effects of decitabine on cardiovascular and respiratory function were investigated in pre-clinical studies and no relevant changes were seen despite higher exposure levels compared to clinical exposure levels.

Decitabine increases fetal hemoglobin expression in patients with thalassemia or sickle cell disease due to the hypomethylation effect.

Relationship between plasma concentration and effect

In non-clinical in vivo tumour models, exposure time was an important factor relative to the efficacy of decitabine; longer exposure via intermittent dosing schedules was more effective than a single higher dose at the same dose intensity in producing an anti-tumoral effect while minimizing toxicity. The superiority of intermittent dosing is due to the additive incorporation of decitabine into DNA during multiple rounds of S-phase in the target cells.

Studies correlating the PK/PD relationship were not performed. However, in an analysis of PD data from an investigator-initiated Phase 1 study of Dacogen for the treatment of subjects with AML or MDS, a dose-dependent decrease in global DNA methylation was observed. Also other clinical studies in patients with haematological malignancies have analysed peripheral blood mononuclear cells (PBMCs) from subjects treated with 5, 10, 15, 20, and 100 mg/m^2 Dacogen per day for methylation of Alu and long interspersed nucleotide element (LINE) DNA sequences. There was a dose dependent linear decrease in methylation of both these markers in samples from subjects that received doses of 5 to 20 mg/m^2 per day. There was no significant additional decrease in methylation above 20 mg/m^2 per day, similar to in vitro observations. The extent of hypomethylation correlated with clinical response.

The phase II study ID03-0180 evaluated three schedules of decitabine in 128 patients with myelodysplasia. Response rate (RR= CR+PR) was assessed after a minimum of 3 cycles of treatment in the three arms:

- Arm A: 10 mg/m^2 IV over 1 hour once a day for 10 consecutive days
- Arm B: 20 mg/m^2 IV over 1 hour once a day for 5 consecutive days
- Arm C: 10 mg/m^2 SC twice a day for 5 consecutive days

The cycles were timed every 4 to 8 weeks depending on recovery. Analysis of PBMCs showed that the 20 mg/m^2 regimen had the greatest extent of hypomethylation and the best CR rate compared to the alternative regimens.

No clinical data have been provided on pharmacodynamic interactions or on genetic differences in PD response.

2.4.4. Discussion on clinical pharmacology

The PK properties of decitabine following 20 mg/m^2 DACOGEN administered daily as a 1-hour IV infusion for 5 consecutive days every 4 weeks were evaluated in 45 patients in 2 Phase II studies and 1 Phase III study In addition, a Phase I radiolabeled mass-balance study was conducted to characterize the PK, metabolism and routes of excretion of 20 mg single DACOGEN dose (^{14}C-decitabine) given as IV infusion over 3 hours in 4 cancer patients. A study in patients using a 3-day, 15 mg/m^2 regimen (DACO-018) was included in the population PK analysis.

The analytical method used for determination of decitabine plasma concentrations (LC/MS/MS) presents adequate performance (e.g., intra-batch accuracy and precision ranged between [-5.50% - 1.33%] and [5.95% - 7.77%], respectively). Moreover, blood sampling was performed using tubes preloaded with tetrahydrouridine (THU) as in vitro inhibitor of plasma cytidine desaminase.

All PK parameters were calculated using conventional non-compartmental methods and actual times of blood sampling.

The steady-state volume of distribution following daily 1 h intravenous infusion of 20 mg/m^2 DACOGEN over 5 days for 4 weeks is 116 L for a 70 kg cancer patient with BSA of 1.73 m^2, indicating distribution of the drug into peripheral tissues. The in vitro plasma protein binding of decitabine is negligible (<1%) and therefore decitabine is unlikely to displace co administered medicinal products from their plasma protein binding. In addition, decitabine has been shown to be a weak inhibitor of P-gp mediated transport in vitro and is therefore also not expected to affect P-gp mediated transport of co-administered medicinal products. Similar observations for the volume of distribution were seen in mice, rats, rabbits, dogs, and monkey, indicating distribution to peripheral tissues.

There is the potential for a drug-drug interaction with other agents which are also activated by sequential phosphorylation (via intracellular phosphokinase activities) and/or metabolized by enzymes implicated in the inactivation of decitabine (e.g., cytidine deaminase, an enzyme for which genetic polymorphism with significant impact on PK and then on toxicity have been described for other drugs such as gemcitabine [Cicollini, J Clin Oncol 2010, Farell, Pharmacogenomics 2011]). Therefore, caution should be exercised if these drugs are combined with Dacogen. Pharmacogenetic evaluation has not been performed in addition to PK and Phase I studies, since DNA samples were not available.

Population pharmacokinetic analysis was performed from PK data obtained in 59 patients (44 males and 15 females). According to the results, the inter-individual variability was 70.6% and 55.7% for central volume and clearance, respectively. Intra-individual variability of decitabine concentrations was moderate (CV=31.6%). The value proposed as intra-individual variability (31.6%) should correspond to residual variability. This value is rather high for pharmacokinetic analysis after IV infusion. It was probably due, at least partly, to the fact that no interoccasion option was used to analyse data from two cycles. Use of interoccasion option is recommended since “ignoring such interoccasion variability (IOV) may result in biased population parameter estimates” [J Pharmacokinet Biopharm. 1993 Dec;21(6):735-50. The importance of modelling interoccasion variability in population pharmacokinetic analyses. Karlsson MO, Sheiner LB.].

Pharmacokinetic - pharmacodynamic (PD) studies have not been conducted with DACOGEN due to the practical difficulties of conducting such studies. The modelled Population PK analyses incorporated subjects representative of the vast majority of the target population (i.e., BSA of 1.6 to 2.5 m^2). These analyses concluded that clearance was proportional to body surface area.

Decitabine metabolism is not mediated by the CYP450 enzyme system but by oxidative deamination. Therefore no formal drug interaction studies have been carried out. The inhibitory and induction potential of decitabine towards human CYP isoenzymes as investigated in vitro, is considered very

limited. In addition, there is no known involvement of CYP in the metabolism of decitabine as shown in the human mass-balance study. As a consequence, CYP-mediated metabolic drug-drug interactions are unlikely. Furthermore, patients in clinical studies were being co-administered a range of other medications and no clinically significant interactions were noted.

Studies in patients with renal or hepatic impairment have not been conducted. The need for dose adjustment in patients with renal hepatic impairment has not been evaluated. Caution should be exercised and patients should be monitored closely.

No dose-response or exposure-response data are available. No data regarding relationship between degree of DNA demethylation and response rate and/or adverse events or between drug exposure and response rate and/or adverse events have been provided.

In addition to its role in DNA demethylation and cytotoxicity, decitabine is likely to have secondary pharmacodynamic effects, including immunosuppressive, antimicrobial, mutagenic, embryotoxic, teratogenic, and tumourigenic effects. These secondary effects are common with other nucleoside analogues used as antiviral and antineoplastic agents.

2.4.5. Conclusions on clinical pharmacology

Overall, the clinical pharmacology of decitabine has been adequately studied.

2.5. Clinical efficacy

2.5.1. Dose response studies

The AML dosing regimen evaluated in the pivotal DACO-016 study and supportive DACO-017 study was 20 mg/m^2 DACOGEN administered as a 1-hour IV infusion daily for 5 consecutive days every 4 weeks.

The AML dosing regimen was initially based on pre-clinical and pharmacologic observations.

Exploratory Studies PCH 97-19 and PCH 95-11 used a 3-Day dosing regimen of DACOGEN, 15 mg/m^2 as a 4-hour IV infusion every 8 hours for 3 consecutive days every 6 weeks (dose intensity= 22.5 mg/m^2 per week), and showed clinical activity in subjects with MDS and AML. These studies led to the development of 2 Phase 3 studies (Study D-0007 in the U.S. and Study EORTC-06011 in Europe) that also evaluated the 3-Day dosing regimen in patients with MDS. Results of D-0007 led to the approval of the 3-Day regimen for the treatment of patients with MDS in the U.S. and 35 other countries.

2.5.2. Main studies

DACO-016

The pivotal Study DACO-016 was a randomized, open-label, multicenter Phase III study of DACOGEN versus patient's choice of treatment (with physician's advice) (treatment choice, TC) of either supportive care or low-dose cytarabine in older patients with AML.

Methods

Study Participants

Patients 65 years of age or older who were newly diagnosed with histologically confirmed de novo or secondary AML and who had a poor- or intermediate-risk cytogenetic profile, ECOG performance status of 0-2, and adequate organ function determined by laboratory evaluation.

Subjects were to be excluded if they had any of the following main conditions: Acute promyelocytic leukemia; any other active systemic malignancies; received previous chemotherapy (except hydroxyurea), including azacitidine, cytarabine or DACOGEN, for any myeloid disorder; was considered a potential candidate for a bone marrow or stem cell transplant within 12 weeks after randomization.

Treatments

DACOGEN was administered at a dose of 20 mg/m^2 by 1-hour intravenous infusion once daily for 5 consecutive days every 4 weeks.

Cytarabine was administered at a dose of 20 mg/m^2 by subcutaneous injection once daily for 10 consecutive days, repeated every 4 weeks.

Subjects could continue on therapy until they were no longer deriving clinical benefit.

Subjects in both two arms above could have received supportive care at the discretion of their treating physician and as defined in the study protocol.

Cycles for the *supportive care group* were defined as successive 4-week intervals beginning with the day of randomization and ending with the date of study treatment discontinuation.

Supportive care specifically excluded surgery, immunotherapy, biologic therapy, radiotherapy (with the exception of palliative radiotherapy), anticancer hormonal therapy, and oral or systemic chemotherapy in which the goal was to either eradicate or slow the progression of the disease. Those therapies considered acceptable included, but were not limited to, treatment with antibiotics and antifungal agents, packed red blood cells or whole blood transfusions, fresh frozen plasma, platelet transfusions, nutritional support (enteral or parenteral), and/or focal external beam radiation given for symptomatic control of pain. Subjects were allowed to receive erythropoietin and darbepoetin during this study; however, use of G-CSF and GM-CSF was restricted to the treatment of severe infection.

Objectives

The primary objective of the pivotal study was to compare the overall survival (OS) in patients 65 years and older who had newly diagnosed de novo or secondary acute myeloid leukemia (AML) and either poor- or intermediate-risk cytogenetics who were randomly assigned to receive either DACOGEN or patient's choice with physician's advice of either supportive care or low-dose cytarabine (Treatment Choice arm [TC]).

The key secondary objective was to compare complete remission rates (CR plus CRp [CR without complete platelet recovery]) and to characterize and compare the incidence and severity of toxicities between treatment arms.

Key tertiary objectives were to compare between treatment arms, event-free survival (EFS), and relapse-free survival (RFS); and to determine the population pharmacokinetics of decitabine.

Outcomes/endpoints

Primary Efficacy: OS was measured from the date of randomization to the date of death from any cause or the last date known to be alive.

Secondary Efficacy: Disease response (CR + CRp) criteria were predefined in the study protocol and based upon review of bone marrow assessments, peripheral blood counts, cytogenetic analyses, evaluation of extramedullary disease, transfusion requirements and clinical judgement.

Tertiary Efficacy: EFS, RFS, and progression-free survival (PFS) were evaluated as tertiary endpoints.

Sample size

The planned sample size was 480 subjects and the targeted number of deaths at the final analysis was 385. The study was designed to detect a 25% reduction in mortality risk (median survival times were assumed to be 6 months for TC arm and 8 months for DACOGEN arm) with at least 80% power while maintaining an overall significance level of 0.05 (2-sided).

Randomisation

485 subjects were randomized. Randomization was stratified for age (<70, 70+ years), cytogenetic risk (intermediate, poor), and ECOG performance status (0-1, 2).The primary analysis of OS was planned when 385 deaths had occurred.

Blinding (masking)

For this open-label study, steps were implemented to minimize potential bias due to unblinded treatment assignments. These included: (1) preselection of the preferred comparator (supportive care or cytarabine) prior to randomization; (2) a central randomization procedure where the next treatment assignment was unknown to all parties prior to randomization; (3) central, blinded outcome assessment of all bone marrow samples used to determine response; (4) independent expert determination of disease response and progression; and (5) no dissemination of ongoing trial information without the prior approval of the Steering Committee in consultation with the DMC.

Statistical methods

OS was calculated as the interval from the date of randomization to the date of death from any cause or the last date the patient was known to be alive.

Survival data were censored at the date of clinical cut off (CCO) on 28 October 2009 (referred to as CCO 2009) for patients who were then known to be alive. Patients lost to follow-up prior to the data CCO were right censored at the last date they were known to be alive. The primary treatment comparison was based on a stratified log-rank test. Stratification factors were to include age (<70, 70+ years), cytogenetic risk (intermediate, poor), ECOG performance status (0-1, 2). The significance level for the final analysis of the primary endpoint was 0.0462 (2-sided) after adjusting for 2 interim analyses. The Kaplan-Meier method was used to estimate the survival function for each treatment arm. The hazard ratio estimate (DACOGEN versus Total TC) and its corresponding 95% CI were obtained by a stratified Cox's regression model using age, baseline cytogenetic risk and baseline ECOG performance status as the strata, and with treatment group as the only covariate. An additional

sensitivity analysis was performed where subjects who received subsequent, potentially disease modifying therapy (DMT) were censored at the first day of the first subsequent therapy.

The incidence of CR + CRp was calculated and compared between 2 treatment arms using Fisher's exact test; this was also done for CRc. Event-free survival was calculated as the interval from the date of randomization to the date of treatment failure, relapse from CR, death from any cause, or the last follow-up examination, whichever occurred first. Progression-free survival was calculated as the interval from the date of randomization to the date of disease progression/relapse or death from any cause, whichever occurred first. Analyses for EFS and PFS were similar to OS. Relapse-free survival was calculated as the interval from the date of CR to the date of relapse, death from any cause, or last follow up examination, whichever occurred first. Kaplan-Meier product limit estimators were used to describe the results.

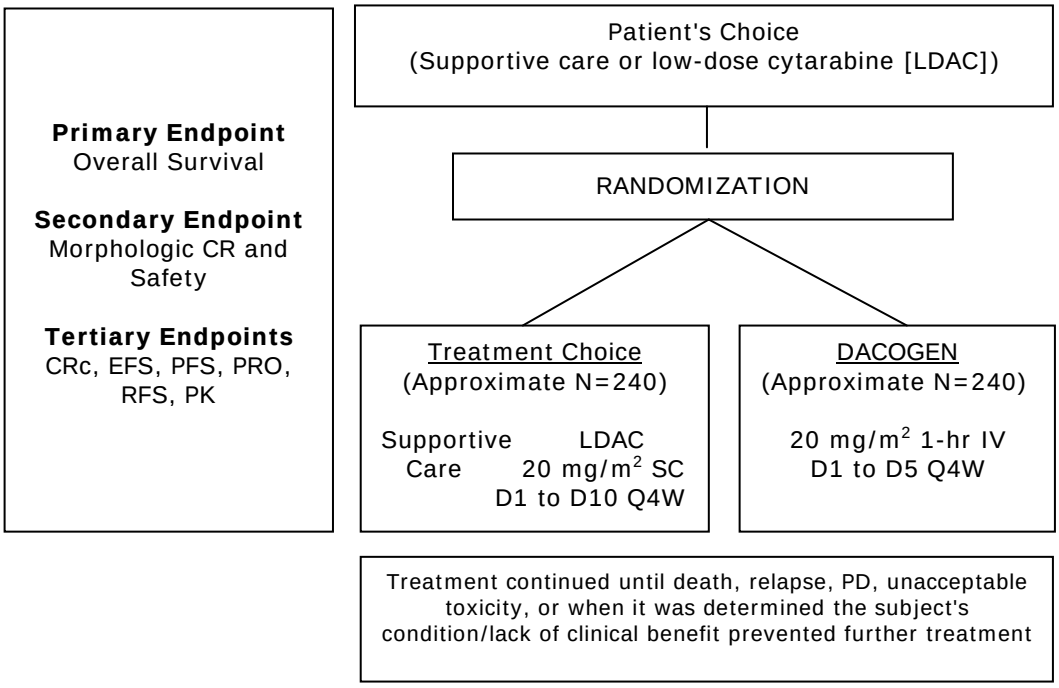
The methods used for the primary OS analysis were applied to the ad hoc analysis of mature survival data (clinical cut off 29 Oct 2010; referred to as CCO 2010). The p-value from this analysis was considered nominal.

Results

Participant flow

At the time of CCO 2009, 46 subjects (9.5%) were ongoing; 15 (6.2%) in the TC arm and 31 (12.8%) in the DACOGEN arm. The number of subjects who discontinued was 439 (90.5%); 228 (93.8%) in the TC arm and 211 (87.2%) in the DACOGEN arm. Progressive disease (PD) was the most common reason attributed by the investigators for discontinuation from treatment. This occurred in 212 (43.7%) subjects overall; 116 (47.7%) in the TC arm and 96 (39.7%) in the DACOGEN arm. At the time of CCO 2010, 10 subjects (2.1%) remained on treatment (7 [2.9%] in the DACOGEN arm and 3 [1.2%] in the TC arm, all in the cytarabine group), and 475 subjects (97.9%) had discontinued study treatment; mostly due to PD (36%) or an AE (33%).

Figure 2: Study DACO-016 Design



CR=complete remission; CRc=cytoge netic CR; D=Day; EFS=event-free survival; hr=hour; IV=intravenously; PD=progressive disease; PFS=progression-free survival; PK=pharmacokinetics; PRO=patient-reported outcomes; Q4W=every 4 weeks; RFS=relapse-free survival; SC=subcutaneously

Recruitment

Study DACO-16 was a multicenter, multinational study conducted in 65 clinical sites throughout Eastern Europe, North America/Australia, Western Europe, and Asia.

The study was initiated the 12 January 2006 (Date of the first study-related procedure/observation recorded as part of the database) and completed the 28 October 2009 (Date of the last subject recorded as part of the database; clinical cut-off date for the primary analysis).

The clinical cut-off for the mature survival and the updated safety analysis date was 29 October 2010.

Conduct of the study

The original protocol, dated 18 July 2005, was never executed. There were a total of 5 protocol amendments. Amendment 1 was implemented before the first subject was enrolled.

Baseline data

Demographic, baseline and baseline disease characteristics are presented in the tables below.

CR=complete remission; CRc=cytoge netic CR; D=Day; EFS=event-free survival; hr=hour; IV=intravenously; PD=progressive disease; PFS=progression-free survival; PK=pharmacokinetics; PRO=patient-reported outcomes; Q4W=every 4 weeks; RFS=relapse-free survival; SC=subcutaneously

Table 16: Demographic and Baseline Characteristics (Study DACO-016) (Intent-to-Treat Analysis Population)

	Treatment Choice (TC)				
	SC (N=28)	**Cytarabine** (N=215)	**Total TC** (N=243)	**DACOGEN** (N=242)	**Total** (N=485)
Age (years)					
N	28	215	243	242	485
Category, n (%)					
<65	0	1 (0.5)	1 (0.4)	3 (1.2)	4 (0.8)
65-69	5 (17.9)	64 (29.8)	69 (28.4)	68 (28.1)	137 (28.2)
70-74	8 (28.6)	66 (30.7)	74 (30.5)	76 (31.4)	150 (30.9)
75-79	8 (28.6)	49 (22.8)	57 (23.5)	65 (26.9)	122 (25.2)
≥80	7 (25.0)	35 (16.3)	42 (17.3)	30 (12.4)	72 (14.8)
Mean (SD)	75.00 (5.340)	73.33 (5.693)	73.53 (5.668)	73.14 (5.242)	73.34 (5.457)
Median	75.00	73.00	73.00	73.00	73.00
Range	(66.0;86.0)	(64.0;91.0)	(64.0;91.0)	(64.0;89.0)	(64.0;91.0)
Gender, n (%)					
N	28	215	243	242	485
Male	20 (71.4)	131 (60.9)	151 (62.1)	137 (56.6)	288 (59.4)
Female	8 (28.6)	84 (39.1)	92 (37.9)	105 (43.4)	197 (40.6)
ECOG performance status, n (%)					
N	28	215	243	242	485
0	7 (25.0)	40 (18.6)	47 (19.3)	42 (17.4)	89 (18.4)
1	12 (42.9)	119 (55.3)	131 (53.9)	140 (57.9)	271 (55.9)
2	9 (32.1)	56 (26.0)	65 (26.7)	60 (24.8)	125 (25.8)

ECOG=Eastern Cooperative Oncology Group, SC=supportive care, SD=standard deviation, TC = patient's choice of treatment with physician's advice

Table 17: Baseline Disease Characteristics (Study DACO-016) (Intent-to-Treat Analysis Population)

	Treatment Choice (TC)				
	SC (N=28)	Cytarabine (N=215)	Total TC (N=243)	DACOGEN (N=242)	Total (N=485)
Type of AML, n (%)					
N	28	215	243	242	485
De novo AML	17 (60.7)	140 (65.1)	157 (64.6)	155 (64.0)	312 (64.3)
Secondary AML	11 (39.3)	73 (34.0)	84 (34.6)	87 (36.0)	171 (35.3)
NAP	0	2 (0.9)	2 (0.8)	0	2 (0.4)
Type of secondary AML, n (%)					
N	11	73	84	87	171
MDS	10 (90.9)	64 (87.7)	74 (88.1)	59 (67.8)	133 (77.8)
Myeloproliferative disorder	1 (9.1)	7 (9.6)	8 (9.5)	16 (18.4)	24 (14.0)
Proven leukemogenic exposure	0	2 (2.7)	2 (2.4)	12 (13.8)	14 (8.2)
FAB Classification, n (%)					
N	28	215	243	242	485
M0	3 (10.7)	18 (8.4)	21 (8.6)	17 (7.0)	38 (7.8)
M1	4 (14.3)	52 (24.2)	56 (23.0)	48 (19.8)	104 (21.4)
M2	13 (46.4)	87 (40.5)	100 (41.2)	102 (42.1)	202 (41.6)
M4	4 (14.3)	34 (15.8)	38 (15.6)	46 (19.0)	84 (17.3)
M4EO	0	1 (0.5)	1 (0.4)	0	1 (0.2)
M5	2 (7.1)	7 (3.3)	9 (3.7)	11 (4.5)	20 (4.1)
M6	0	5 (2.3)	5 (2.1)	8 (3.3)	13 (2.7)
M7	1 (3.6)	1 (0.5)	2 (0.8)	1 (0.4)	3 (0.6)
NAP	1 (3.6)	4 (1.9)	5 (2.1)	3 (1.2)	8 (1.6)
Unknown	0	6 (2.8)	6 (2.5)	6 (2.5)	12 (2.5)
Blasts in bone marrow (%)					
N	28	213	241	241	482
Category, n (%)					
<20	2 (7.1)	6 (2.8)	8 (3.3)	4 (1.7)	12 (2.5)
20-30	5 (17.9)	53 (24.9)	58 (24.1)	65 (27.0)	123 (25.5)
>30-50	10 (35.7)	64 (30.0)	74 (30.7)	67 (27.8)	141 (29.3)
>50	11 (39.3)	90 (42.3)	101 (41.9)	105 (43.6)	206 (42.7)
Mean (SD)	48.81 (25.053)	49.69 (23.290)	49.58 (23.449)	50.31 (23.826)	49.95 (23.616)
Median	40.25	46.00	45.00	46.60	45.95
Range	(12.0;100.0)	(0.0;100.0)	(0.0;100.0)	(3.0;100.0)	(0.0;100.0)
Cytogenetic classification of risk	28	214	242	241	483
Intermediate risk	20 (71.4)	134 (62.6)	154 (63.6)	152 (63.1)	306 (63.4)
Poor risk	8 (28.6)	79 (36.9)	87 (36.0)	87 (36.1)	174 (36.0)
NAV	0	0	0	2 (0.8)	2 (0.4)
UNK	0	1 (0.5)	1 (0.4)	0	1 (0.2)

AML=acute myeloid leukemia, FAB=French-American-British; MDS=myelodysplastic syndromes; NAP=not applicable; NAV=not available; SC=supportive care; SD=standard deviation, TC=patient's choice of treatment with physician's advice; UNK=unknown

Numbers analysed

At the time of the primary analysis CCO 2009, 396 deaths had occurred. The table below summarises the subjects included in the analysis datasets. The intent-to-treat (ITT) population was used for the efficacy endpoint analyses. The safety population was used for all safety analyses.

Table 18: Number of Subjects by Population (Study DACO-016: All Randomized Subjects Analysis Set)

	Treatment Choice (TC)				
	SC	**Cytarabine**	**Total TC**	**DACOGEN**	**Total**
	(N=28)	(N=215)	(N=243)	(N=242)	(N=485)
Analysis Population	n (%)	n (%)	n (%)	n (%)	n (%)
Intent-to-Treat	28 (100)	215 (100)	243 (100)	242 (100)	485 (100)
Per-protocol	16 (57.1)	177 (82.3)	193 (79.4)	203 (83.9)	396 (81.6)
Safety	28 (100)	209 (97.2)	237 (97.5)	238 (98.3)	475 (97.9)

SC=supportive care, TC=patient's choice of treatment with physician's advice

Note: Percentages calculated with the number of subjects in each group as denominator.

Note: 1 subject in the TC arm indicated a pre randomization treatment choice of cytarabine, but received only supportive care

Outcomes and estimation

At the time of CCO 2009, 396 total deaths had occurred (199 in the TC arm and 197 in the DACOGEN arm). The median OS in the ITT population was 5.0 months (95% CI: 4.3, 6.3) in the TC arm and 7.7 months (95% CI: 6.2, 9.2) in the DACOGEN arm, which represents a 2.7-month difference or a 54% improvement over TC. The estimated hazard ratio (HR; DACOGEN arm/ TC arm) was 0.85 (95% CI: 0.69, 1.04) representing a 15% reduction in mortality risk for Dacogen relative to TC. The p-value from the stratified logrank test was 0.1079.

A summary of efficacy results is enclosed below in the table below. Results for the analysis of mature data are from the CCO 2010; all other results are from CCO 2009.

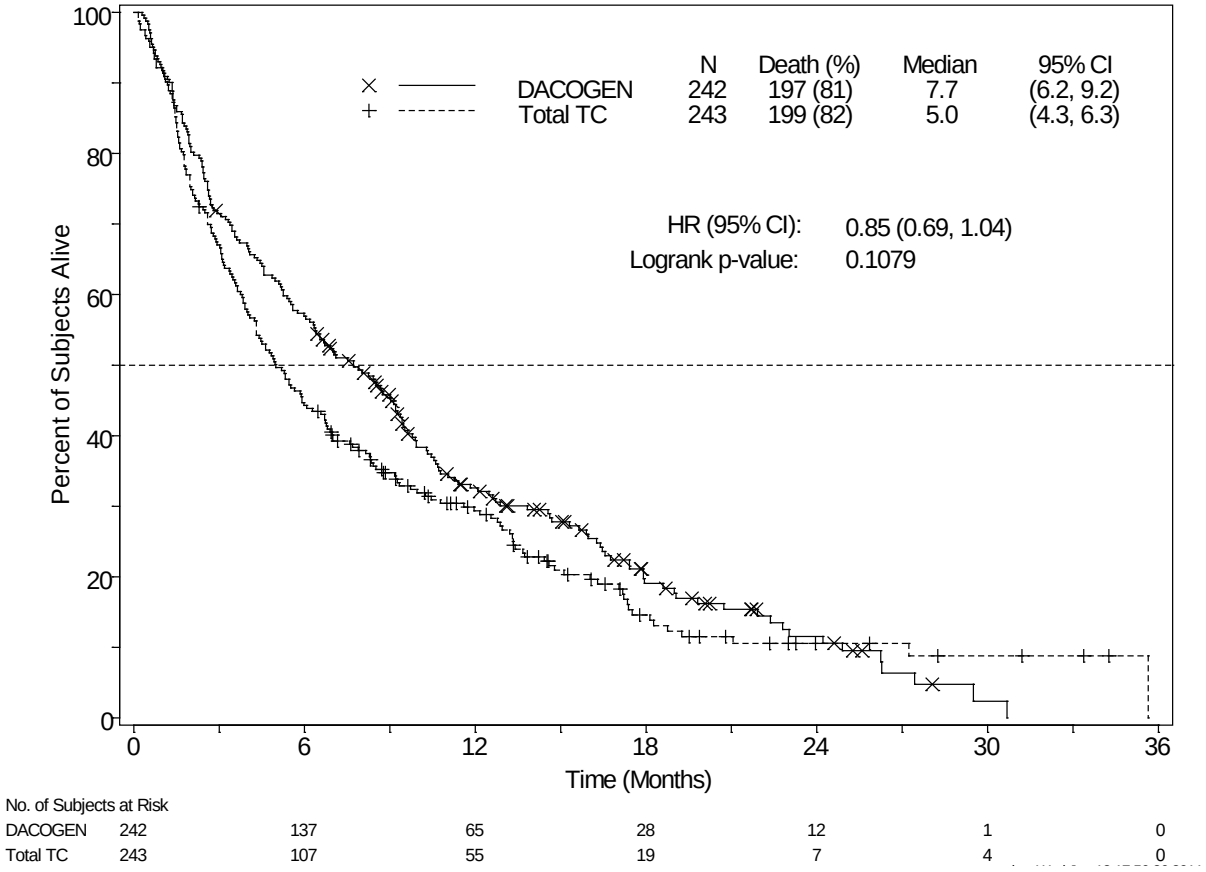
Table 19: DACO-016 Summary of Efficacy Results (ITT)

	TC (N=243)	DACOGEN (N=242)	
	Median (95% CI)	Median (95% CI)	HR (95% CI); p-value
Overall survival (m)			
Primary analysis	5.0 (4.3, 6.3)	7.7 (6.2, 9.2)	0.85 (0.69, 1.04); 0.1079
Censored for the use of DMT	5.3 (4.3, 6.7)	8.5 (6.5, 9.5)	0.80 (0.64, 0.99); 0.0437
Excluding subjects who received HMA	4.5 (3.8, 5.5)	7.9 (6.0, 9.3)	0.77 (0.62, 0.94); 0.0109
Analysis of mature data	5.0 (4.3, 6.3)	7.7 (6.2, 9.2)	0.82 (0.68, 0.99); 0.0373
Censored for the use of DMT	5.3 (4.3, 6.7)	8.6 (6.5, 9.5)	0.79 (0.65, 0.98); 0.0295
Excluding subjects who received HMA	4.4 (3.7, 5.5)	7.9 (6.0, 9.3)	0.73 (0.60, 0.88); 0.0014
EFS, PFS, RFS (m)			
EFS	2.1 (1.9, 2.8)	3.5 (2.5, 4.1)	0.75 (0.62, 0.90); 0.0025
PFS	2.1 (1.9, 3.1)	3.7 (2.7, 4.6)	0.75 (0.62, 0.91); 0.0031
RFS			
in subjects with CR	6.7 (2.9, 13.4)	8.3 (4.6, 11.4)	---
in subjects with CRc	---	---	---
Clinical Response	n **(%)**	n **(%)**	OR (95% CI); p value

CR + CRp	19 (7.8)	43 (17.8)	2.5 (1.40, 4.78); 0.0011
CRc	3/41 (7.3)	4/40 (10)	1.4 (0.22, 10.24); 0.7123
Time to and Duration of Response (m)	Median (95% CI)	Median (95% CI)	
Time to best response (CR or CRp)	3.7 (2.8, 4.6)	4.3 (3.8, 5.1)	
Duration of response (CR or CRp)	12.9 (4.2, NE)	8.3 (6.2, 11.4)	

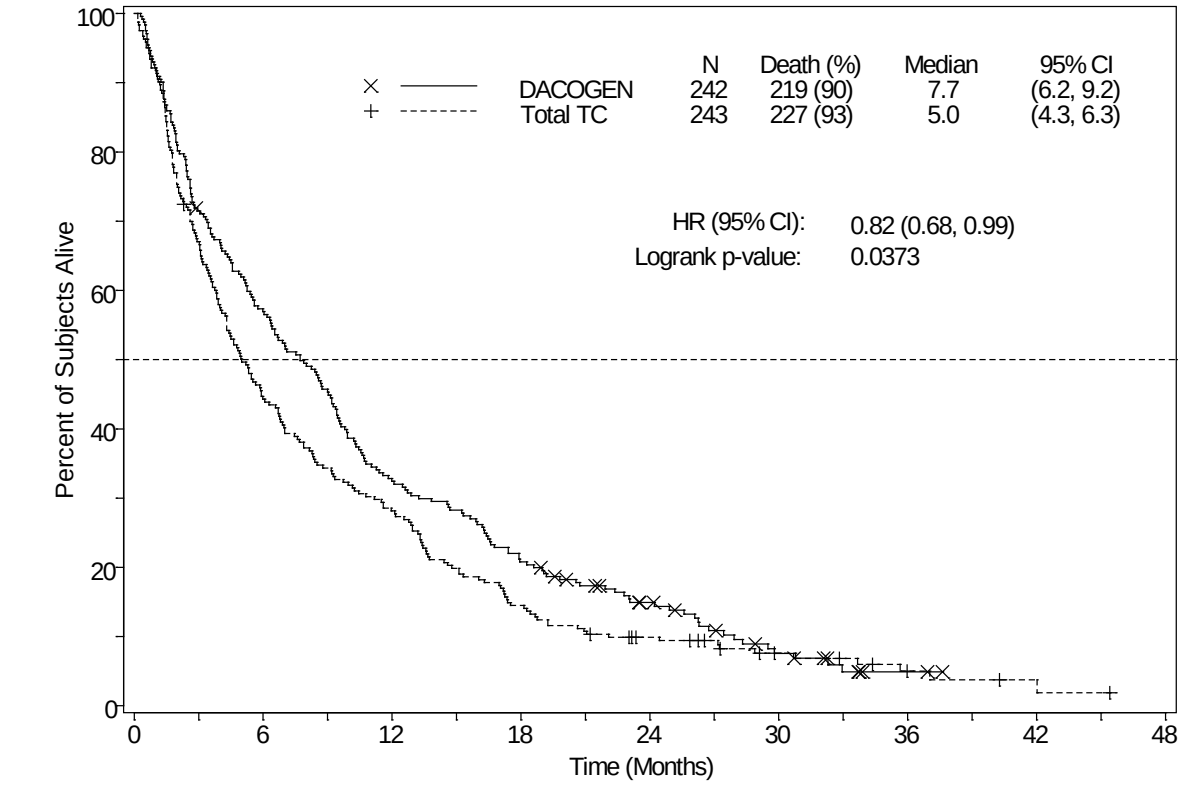
CI=confidence interval; CR=complete remission; CRc=cytogenetic CR, based on subjects with cytogenetic abnormalities at baseline and with at least 1 post-baseline cytogenetic assessment; CRp=CR with incomplete platelet recovery; DMT=disease modifying therapy; EFS=event-free survival; HMA=hypomethylating agent; NE=not estimable; OR=odds ratio; PFS=progression-free survival; RFS=relapse-free survival m=months

Figure 3: Overall Survival (Study DACO-016: Intent-to-Treat Analysis Set)



In an analysis with an additional 1 year of mature survival data, the effect of Dacogen on overall survival demonstrated a clinical improvement compared to the TC arm (7.7 months vs. 5.0 months, respectively, hazard ratio = 0.82, 95% CI: 0.68, 0.99, nominal p-value = 0.0373, Figure 4.

Figure 4: Analysis of mature overall survival data (Study DACO-016: Intent-to-Treat population).

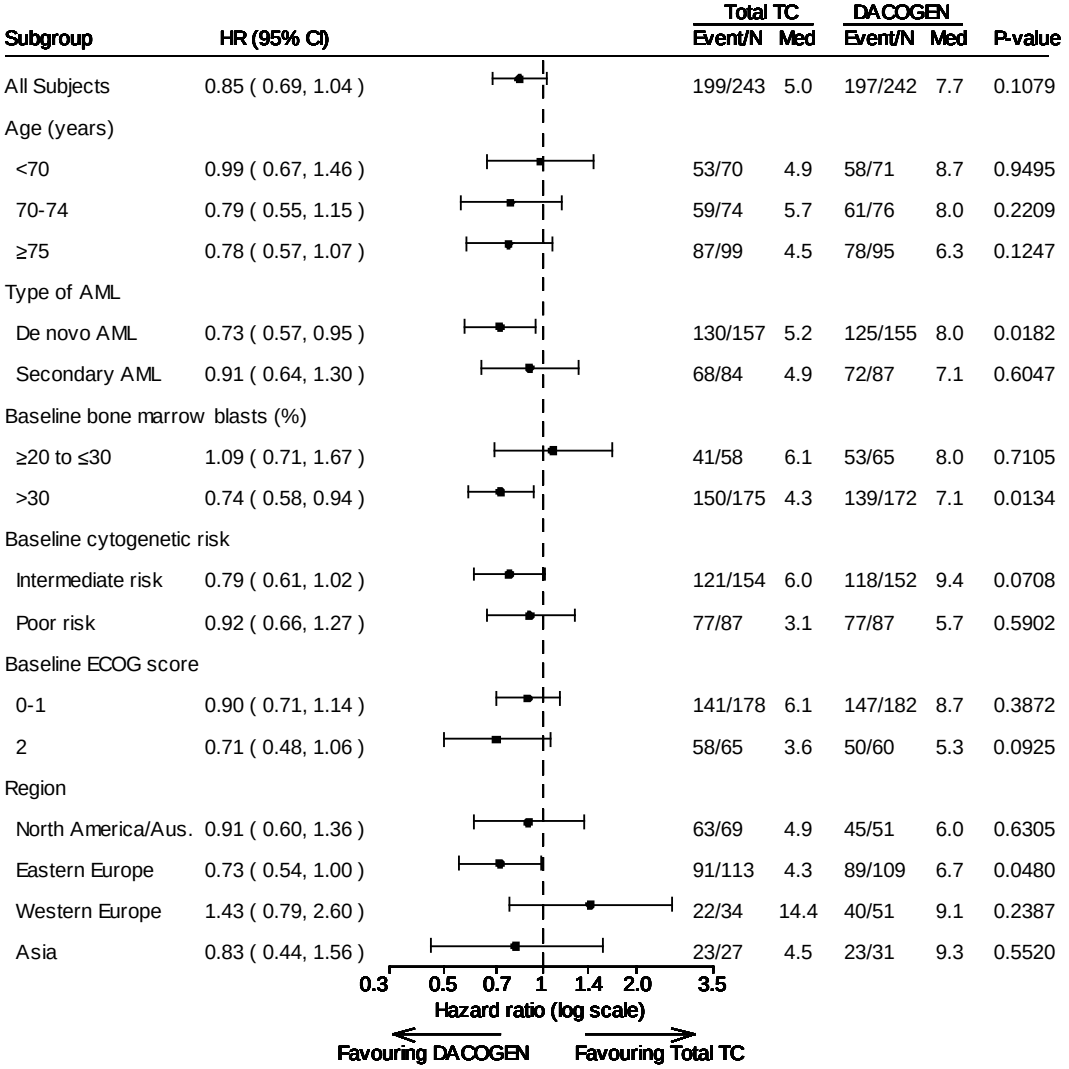


No. of Subjects at Risk

DACOGEN	242	137	78	50	28	11	2	0	0
Total TC	243	107	68	35	20	10	4	2	0

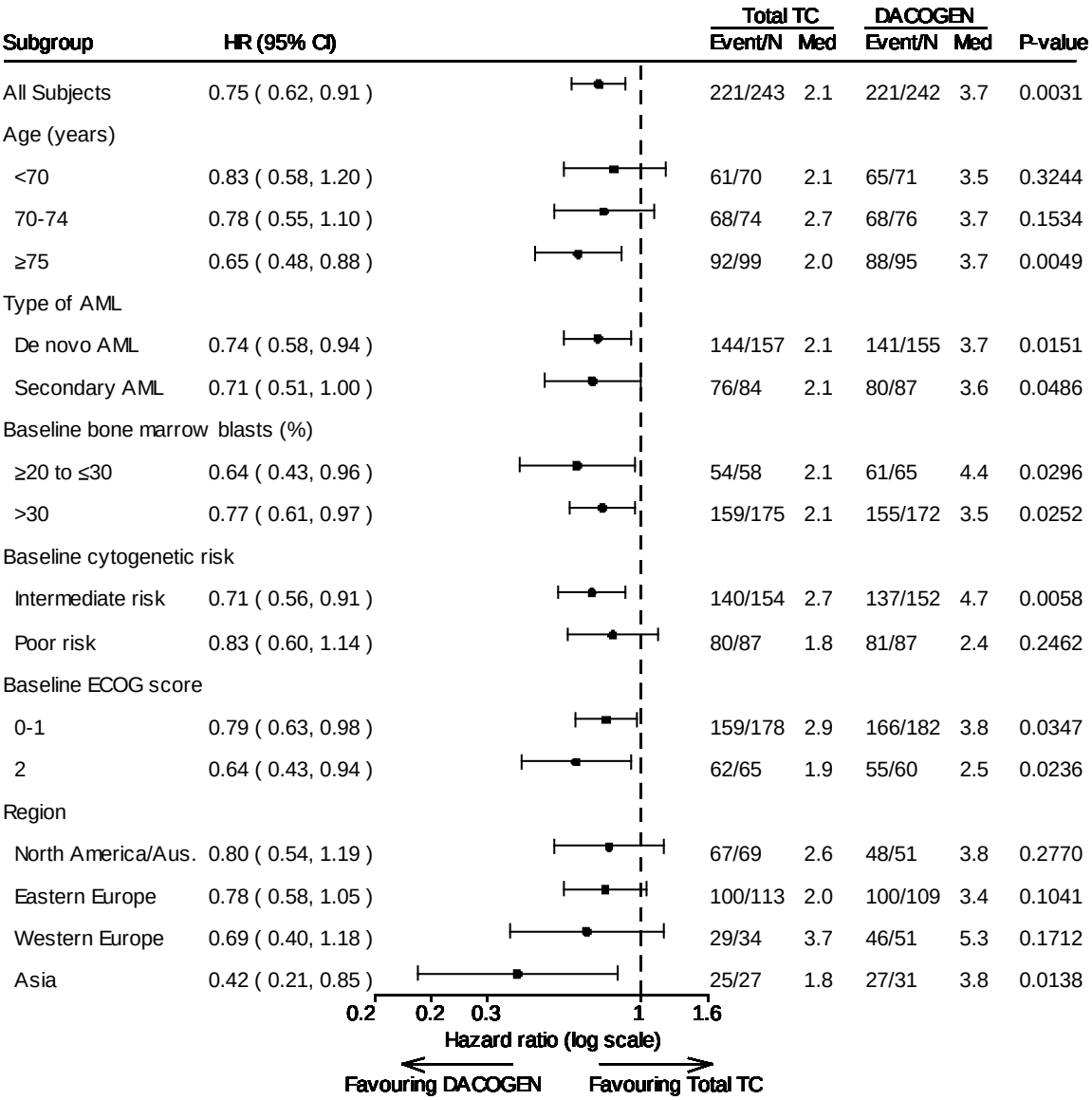
Ancillary analyses

Figure 5: Overall Survival Subgroup Analysis (CCO 2009) (Study DACO-016: Intent-to-Treat Analysis Set)



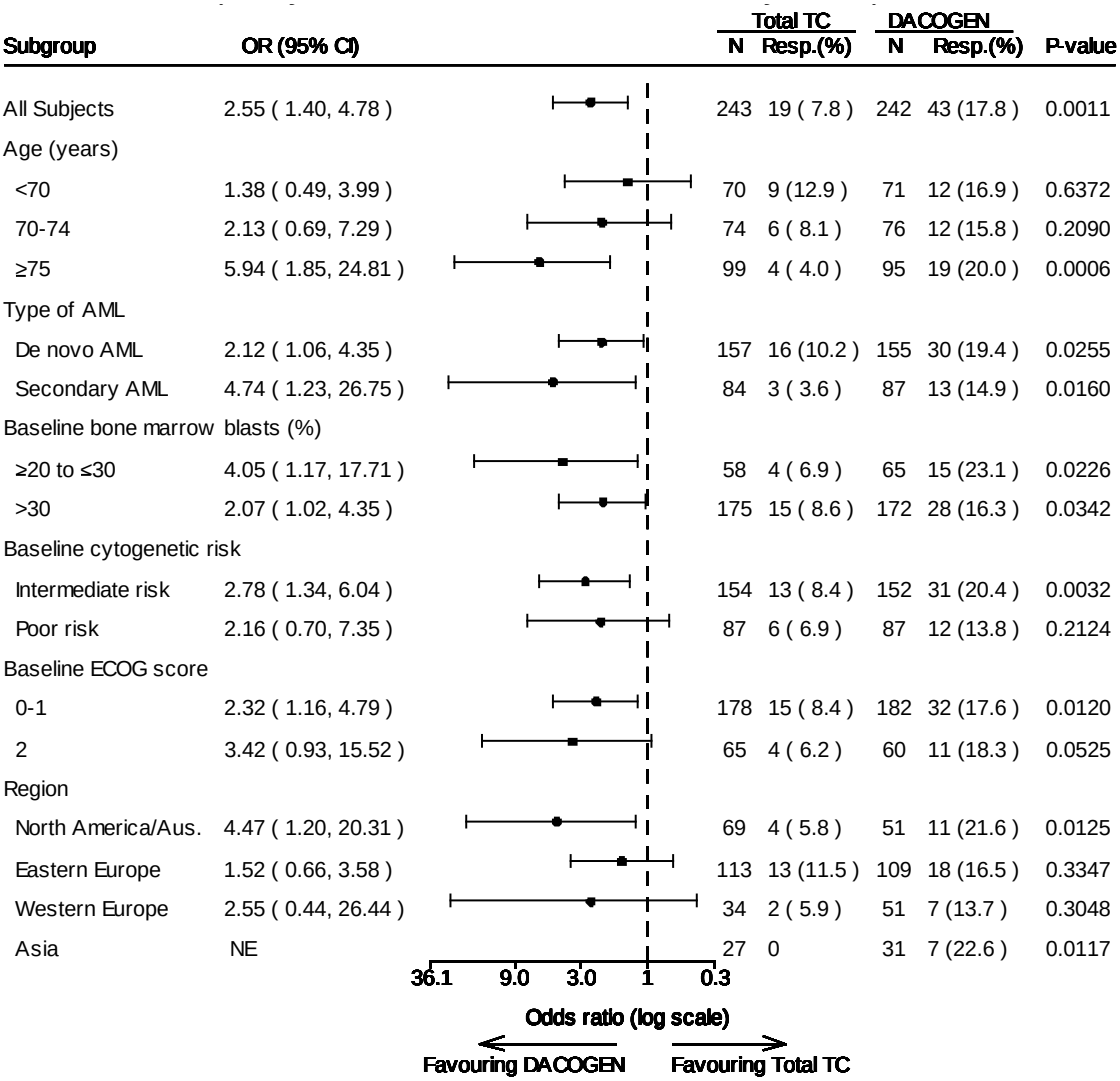
AML = acute myeloid leukemia, Aus. = Australia, CI = confidence interval, ECOG = Eastern Cooperative Oncology Group, HR = hazard ratio, Med = median (months), TC = patient's choice of treatment with physician's advice
Note: p-value is based on two sided log-rank test stratified by age, cytogenetic risk, ECOG performance status.

Figure 6: Progression-Free Survival Subgroup Analysis (Study DACO-016) (Intent to Treat Analysis Population)



AML = acute myeloid leukemia, Aus. = Australia, CI = confidence interval, ECOG = Eastern Cooperative Oncology Group, HR = hazard ratio, Med = median (months), TC = patient's choice of treatment with physician's advice
Note: p-value is based on two sided log-rank test stratified by age, cytogenetic risk, ECOG performance status.

Figure 7: Response Subgroup Analysis of Subjects With Complete Remission or Complete Remission With Incomplete Platelet Recovery (Study DACO-016) (Intent-to-Treat Analysis Population)



AML = acute myeloid leukemia, Aus. = Australia, CI = confidence interval, ECOG = Eastern Cooperative Oncology Group, NE = not estimable, OR = odds ratio, Resp. = responder, TC = patient's choice of treatment with physician's advice
Note: p-value is based on two-sided Fisher's exact test.
Note: odds ratio plot is flipped over the y-axis.

Summary of main study

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 20: Summary of Efficacy for trial DACO-016

Title: Randomized Phase 3 Trial of Decitabine Versus Patient's Choice with Physician's Advice of Either Supportive Care or Low-dose Cytarabine for the Treatment of Older Patients with Newly Diagnosed Acute Myeloid Leukemia	
Study identifier	DACO-016

Design	Randomized, open-label, multicenter Phase III study of decitabine versus patient's choice of treatment (with physician's advice) of either supportive care or low-dose cytarabine		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		Once daily for 5 consecutive days every 4 weeks, Subjects could continue to receive treatment until they were no longer deriving clinical benefit not applicable not applicable
Hypothesis	Superiority		
Treatments groups	Arm A:TC		Patient's choice with physician's advice of either supportive care OR 20 mg/m2 cytarabine given subcutaneously once daily for 10 consecutive days repeated every 4 weeks. Randomised n=243
	Arm B: Decitabine		20 mg/m2 decitabine as a 1-hour intravenous infusion once daily for 5consecutive days repeated every 4 weeks. Randomised n=242
Endpoints and definitions	Primary endpoint	OS	Overall Survival Calculated as the interval from the date of randomization to the date of death from any cause or the last date known to be alive.
	Secondary endpoint	CR+CRp	Morphologic Complete Remission (CR) + CR without platelet recovery (CRp) rate
	Terciary endpoint	CRc	Cytogenetic Complete Remission (CRc)
	Terciary endpoint	EFS	Event-free survival (EFS)
	Terciary endpoint	PFS	Progression-free survival (PFS)
	Terciary endpoint	QoL	Overall QoL score (from the EORTC QLQ-C30)
	Terciary endpoint	Fatigue QoL	Fatigue subscale score (from the EORTC QLQ-C30)
	Terciary endpoint	Nights hopitalised	Nights hospitalized (for medical or treatment reasons)
	Terciary endpoint	RFS	Relapse-free survival (for subset of patients who achieve CR)
Database lock	28 October 2009 (analysis of mature OS data are from the clinical cut off of 29 Oct 2010)		
Results and Analysis			
Analysis description	**Primary Analysis**		

Analysis population and time point description	Intent to treat -Bone marrow aspirate/biopsy samples were obtained at baseline, within 7 days prior to every second cycle beginning at Cycle 3, and at the end of study. -Cytogenetic assessments were performed at baseline, then for subjects with a baseline cytogenetic abnormality and with a documented CR, at the end of the first and third cycles after initial documentation of CR. -Hematology assessments from peripheral blood were performed at baseline, within 4 days before the first dose, then biweekly during the study, and at the end of study.		
Descriptive statistics and estimate variability	Treatment group	TC	Decitabine
	Number of subject (N)	243	242
	OS (median in months)	5.0	7.7
	95% CI	(4.3, 6.3)	(6.2, 9.2)
	Mature OS (median in months)	5.0	7.7
	95% CI	(4.3, 6.3)	(6.2, 9.2)
	CR+CRp (number of subjects/percentage)	19 subjects (7.8%)	43 subjects (17.8%)
	variability statistic	--	--
	CRc (number of subjects/Total no. subjects with cytogenetic abnormalities at baseline and with at least 1 post-baseline cytogenetic assessment) (percentage)	3/41 subjects (7.3%)	4/40 subjects (10%)
	variability statistic	--	--
	EFS (Kaplan-Meier product limit Estimators in months)	2.1	3.5
	95% CI	(1.9, 2.8)	(2.5, 4.1)
	PFS (Kaplan-Meier product limit Estimators in months)	2.1	3.7
	95% CI	(1.9, 3.1)	(2.7, 4.6)

	QoL (change from baseline > -10 point at cycle 3)	68.5% (N=108)	70.8% (N=113)
	variability statistic	--	--
	Fatigue QoL (change from baseline > -10 point at cycle 3)	63.9% (N=108)	63.7% (N=113)
	variability statistic	--	--
	% of Nights Hospitalised Relative to Total Treatment Duration (mean)	43.2% (N=182)	42.1% (N=191)
	SD	(33.58)	(30.86)
	RFS (Kaplan-Meier product limit Estimators in months)	6.7 (N=18)	8.3 (N=38)
	95% CI	(2.9, 13.4)	(4.6, 11.4)

Effect estimate per comparison	Primary endpoint: OS (CCO 2009)	Comparison groups	Decitabine vs TC
		Hazard ratio (HR)	0.85
		(95% CI)	(0.69, 1.04)
		P-value	0.1079 (significance level of 0.0462)
	Primary endpoint: OS (mature data CCO 2010)	Comparison groups	Decitabine vs TC
		Hazard ratio (HR)	0.82
		(95% CI)	(0.68;0.99)
		P-value	0.0373 (nominal)
	Secondary endpoint: CR+CRp	Comparison groups	Decitabine vs TC
		Odds ratio (OR)	2.5
		(95% CI)	(1.40;4.78)
		P-value	0.0011 (significance level of 0.05)
Notes			

Analysis performed across trials (pooled analyses and meta-analysis)

None

Clinical studies in special populations

None

Supportive study

Study DACO-017 was a multicenter, single-arm, Phase 2 study of DACOGEN as front-line therapy in older patients with AML. Patients at least 60 years of age with newly diagnosed histologically confirmed de novo or secondary AML and who had poor- or intermediate-risk cytogenetics at baseline were eligible. Eligible subjects had an ECOG performance status of 0 to 2 and were considered to have adequate renal and hepatic function by laboratory evaluation.

Treatment with DACOGEN consisted of 20 mg/m^2 DACOGEN administered as a 1-hour IV infusion daily for 5 consecutive days every 4 weeks.

The primary efficacy endpoint was morphologic CR. Key secondary efficacy endpoints included OS, CRc, complete remission with incomplete blood count recovery (CRi), CRp, PR, time to and duration of CR, EFS, and RFS.

A morphologic CR was achieved in 13 of 55 subjects (23.6%) and 1 additional subject achieved a CRi (CR with incomplete blood count recovery). The median time to CR was 4.1 months. Five of the 13 subjects with CR had relapsed as of the CCO and the median duration of response was 18.2 months.

The median OS was 7.6 months (95% CI: 5.7, 11.5).

Overall, these results are consistent with what observed in the pivotal study.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The Applicant has provided results mainly taken from one single pivotal study (DACO-016) and one supportive phase II study (DACO-017) where decitabine was administered as front-line therapy in older patients with AML.

The pivotal study was a randomized, open-label, phase III study designed to compare decitabine with patient's choice with physician's advice of supportive care or low dose of cytarabine (treatment's choice, TC).

Baseline characteristics were comparable in both studies; however, patients were younger in the supportive study. The included patient population was broad and heterogeneous.

The dosing regimen was the same in both two studies.

In the pivotal study, the primary efficacy endpoint was OS and the key secondary objective was CR.

In the supportive study, morphologic CR was the primary endpoint. Key secondary efficacy endpoints included OS, CRc, complete remission with incomplete blood count recovery (CRi), CRp, PR, time to and duration of CR, EFS, and RFS.

Efficacy data and additional analyses

An increase in median survival of 2.7 months was considered as clinically meaningful in such a particular setting.

Potential factors that could have contributed to the primary analysis not reaching statistical significance include an i) imbalance in subgroup characteristics, ii) subsequent therapy usage, iii) data censoring in the period following the median of the curve.

Therefore, 2 sensitivity analyses were performed.

1) A sensitivity analysis of OS that censored data at the time subjects began receiving subsequent disease modifying therapy (DMT), defined as induction chemotherapy, azacitidine, and DACOGEN, showed a 20% reduction in the risk of death for subjects in the DACOGEN arm The median (95% CI) OS was 5.3 (4.3, 6.7) months in the TC arm and 8.5 (6.5, 9.5) months in the DACOGEN arm, and the HR was 0.80 (p=0.0437).

2) A further sensitivity analysis excluded subjects who received a hypomethylating agent as subsequent DMT. In this analysis, a 23% reduction in mortality risk was observed with DACOGEN treatment compared with TC. The median OS in months (95% CI) was 4.5 (3.8, 5.5) in the TC group and 7.9 (6.0, 9.3) in the DACOGEN group. The HR (95% CI) was 0.77 (0.62, 0.94); p-value= 0.0109

In addition, an analysis of more mature survival data collected 1 year after the initial CCO was provided. The results were consistent with those of the primary study analysis. The median OS (95% CI) was 5.0 (4.3, 6.3) months in the TC arm and 7.7 (6.2, 9.2) months in the DACOGEN arm. The estimated HR was 0.82 (95% CI: 0.68, 0.99), nominal p-value=0.0373; representing a slightly larger (18%) reduction in the risk of mortality with DACOGEN treatment.

2.5.4. Conclusions on the clinical efficacy

Taken into consideration the data submitted, the CHMP considered that the indication of decitabine should be restricted to the treatment of adult patients aged 65 years and above with newly diagnosed de novo or secondary AML according to the WHO classification who are not considered candidates for standard induction chemotherapy. This proposed age limit is considered acceptable.

2.6. Clinical safety

Supporting safety data come from 1,114 subjects from 2 studies (Studies DACO-016 and DACO-017) in patients with AML (integrated AML population or AML set) and 4 studies in patients with MDS (integrated MDS population or MDS set) (Studies DACO-020, ID03-0180, D-0007, EORTC-06011).

Patient exposure

The AML set included the two open-label studies DACO-016 and DACO-017. The number of patients included in study DACO-017 was small; however, these patients contributed for about 19% of the patients exposed to Dacogen, included in AML data set. Patients in study DACO-017 may have dose delay and/or 25% dose reduction. Moreover, patients included in the two studies DACO-016 and DACO-017 were different in term of duration of exposure.

Therefore, the two studies data could not *a priori* be pooled and the results from the provided integrated AML studies should be taken with caution.

Only data collected in study DACO-016 are relevant to provide a comprehensive assessment of the safety of DACOGEN in the claimed indication.

The applicant also presented the MDS set including 4 MDS studies (DACO-020 and ID03-0180; D-0007 and EORTC-06011). Despite MDS and AML can be considered a continuum of the same myeloid

malignancy, the two diseases are too different in term of prognosis and patient characteristics. Moreover population in MDS set was not homogenous.

Adverse events

The most frequently observed adverse events in the DACOGEN group of the AML studies (occurring in ≥ 30% of all subjects) were pyrexia, thrombocytopenia, anemia, nausea, febrile neutropenia, neutropenia, and diarrhea.

In DACO-16 study report, TEAEs occurring in at least 5% of patients were provided. In the integrated AML studies, the limit is 10%. For serious events the limit is 2%.

All serious AEs >5% reported more frequently in Dacogen group than in Cytarabine group, in study DACO-016 are included in SmPC section 4.8.

For the integrated AML studies, Grade 3 and 4 adverse events occurred in nearly all subjects in both the DACOGEN and cytarabine treatment groups.

The incidences of Grade 3 and 4 adverse events for subjects in the DACOGEN treatment group were slightly higher than those of the cytarabine group.

The most frequently observed Grade 3 and 4 adverse events were related to myelosuppression, consistent with experience in patients with AML. The percentages of AES in relation with myelosuppression are higher in DACOGEN treatment group than in cytarabine treatment group.

In the AML studies, DACOGEN-treated subjects had higher rates of most infections than the cytarabine groups, including Grade 3 or 4 infections. Febrile neutropenia, pneumonia, and urinary tract infection were the most common infections.

Adverse events of special interest: In the integrated AML studies, adverse events of special interest were experienced by 89% of subjects in the cytarabine group and 92% in the DACOGEN group:

- For all subjects in the AML studies DACO-016 and DACO-017 who received DACOGEN, 73% experienced a myelosuppression event; 69% experienced a Grade 3 or 4 event. Leukopenia occurred in 13% and 20% of subjects, respectively in the cytarabine and DACOGEN treatment groups.
- In the AML studies, Decitabine -treated subjects had higher rates of most infections than the cytarabine groups, including Grade 3 or 4 infections. Febrile neutropenia, pneumonia, and urinary tract infection were the most common infections.
- In the integrated AML population, bleeding events occurred with similar frequency in DACOGEN treated subject (11%) and cytarabine-treated subjects (13%). Gastrointestinal hemorrhage occurred more frequently with cytarabine treatment, while intracranial bleeding was reported more frequently with Decitabine treatment.
- In the AML population, skin eruptions occurred more frequently in DACOGEN-treated subjects. These were almost exclusively low-grade.
- For the AML population, Cardiac Rhythm abnormalities were reported by 20% in the cytarabine group, and 19% in the Decitabine group. Tachycardia was experienced by similar percentages of subjects in the cytarabine (5%) and Decitabine (6%) groups. In the integrated AML studies, neurological events were experienced by 3% of subjects in the cytarabine treatment group and 4%

in the DACOGEN group. These events were primarily intracranial haemorrhage experienced by 1 subject in the cytarabine group and 6 subjects in the DACOGEN group.

The adverse reactions identified with Dacogen are presented in the table below.

Table 21: Adverse drug reactions identified with Dacogen.

System Organ Class	Frequency (all Grades)	Adverse Drug Reaction	Frequency	
			All Grades[a] (%)	Grades 3-4[a] (%)
Infections and infestations	Very common	pneumonia*	24	20
		urinary tract infection*	15	7
	Common	septic shock*	6	4
		sepsis*	9	8
		sinusitis	3	1
Blood and lymphatic disorders	Very common	febrile neutropenia*	34	32
		neutropenia*	32	30
		thrombocytopenia[b*]	41	38
		anaemia	38	31
		leukopenia	20	18
	Uncommon	pancytopenia*	<1	<1
Immune system disorders	Common	hypersensitivity including anaphylactic reaction[c]	1	<1
Nervous system disorders	Very common	headache	16	1
Respiratory, thoracic and mediastinal disorders	Very common	epistaxis	14	2
Gastrointestinal disorders	Very common	diarrhoea	31	2
		vomiting	18	1
		nausea	33	<1
	Common	stomatitis	7	1
Skin and subcutaneous tissue disorders	Uncommon	acute febrile neutrophilic dermatosis (Sweet's syndrome)	< 1	NA
General disorders and administration site conditions	Very common	pyrexia	48	9

[a] Worst National Cancer Institute Common Terminology Criteria for Adverse Events Grade
[b] Including haemorrhage associated with thrombocytopenia, including fatal cases
[c] Including preferred terms hypersensitivity, drug hypersensitivity, anaphylactic reaction, anaphylactic shock, anaphylactoid reaction, anaphylactoid shock.
*Includes events with a fatal outcome
NA=Not applicable

Serious adverse event/deaths/other significant events

Treatment-emergent serious adverse events were more common in DACOGEN-treated subjects (80%) than in subjects treated with cytarabine (73%) due to higher rates of Infections and Infestations, Blood and Lymphatic System Disorders, and Respiratory, Thoracic and Mediastinal Disorders. Moreover in Dacogen group, except for "vascular disorders" and "nervous system disorders", all the percentage of serious adverse events are higher than in cytarabine group.

For the AML studies, Grade 3 or 4 serious adverse events were experienced by 34% of subjects in the Supportive Care group, 63% in the cytarabine group, and 73% in the Decitabine group. System organ classes with the highest incidences of Grade 3 or 4 serious adverse events were Infections and Infestations (Supportive Care: 28%; cytarabine: 30%; Decitabine: 47%) and Blood and Lymphatic System Disorders (Supportive Care: 0%; cytarabine: 28%; Decitabine: 35%). The most frequently reported serious adverse events were febrile neutropenia (Supportive Care: 0%; cytarabine: 15%; Decitabine: 24%) and pneumonia (Supportive Care: 10%; cytarabine: 14%; Decitabine: 18%). In the integrated AML studies, the percentages of early deaths were comparable between the cytarabine (8.7%) and Decitabine (8.5%) treatment groups.

In the integrated AML studies, the percentages of early deaths were comparable between the cytarabine (8.7%) and DACOGEN (8.5%) treatment groups. In the integrated AML studies, the percentages of subjects who died during treatment or within 30 days after the last dose of study drug were 30.3% in the cytarabine treatment group and 32.8% in the DACOGEN treatment groups. The Applicant states that more deaths were attributed to cardiac causes in DACOGEN-treated subjects in DACO-016 (13 subjects who received DACOGEN versus 6 who received cytarabine), more than 60% of these cases were from a single country (Poland), with 6 cases from a single site.

Laboratory findings

A high percentage of subjects entered the study with Grade 3 or 4 neutropenia (absolute neutrophil count [ANC]; 62% [313/506]) and thrombocytopenia (platelet count; 47% [229/486]). The majority of subjects in all treatment groups entered the study with Grade 2 anemia (Hb). Baseline WBC counts varied, reflecting both leukopenia and leukocytosis. In the AML study subjects, high percentages of subjects experienced Grade 3 or 4 laboratory test results at some time during treatment. In the Decitabine group, 89% (236/266) of subjects reported Grade 3 or 4 neutropenia (ANC).

In the DACOGEN group, Grade 4 laboratory test results were reported for no more than 1% of subjects. Grade 3 laboratory test results were reported for no more than 1% of subjects with the exceptions of ALT (3% [9/278]), and hyperglycemia (10% [28/284]).

Two (1%) subjects shifted from normal (Grade 0 or 1) creatinine values at baseline to Grade 3 during treatment. No subject experienced a Grade 4 creatinine result in any treatment group.

In the cytarabine group, there were no Grade 4 test results for any chemistry analyse. Grade 3 test results were reported for no more than 3% of subjects except hyperglycemia (6% [11/196]).

Safety in special populations

In the DACOGEN treatment group, there was a higher incidence of treatment discontinuation due to adverse events in women compared to men (43% versus 32%).

Safety related to drug-drug interactions and other interactions

No formal drug interaction studies with decitabine have been conducted.

Discontinuation due to adverse events

In the integrated AML studies, treatment-emergent adverse events leading to discontinuation occurred more frequently in the cytarabine group than the DACOGEN group. Drug-related adverse events leading to discontinuation were rare and most often due to the expected complications of

myelosuppression and infection. Serious adverse events and drug-related serious adverse events leading to discontinuation occurred at similar rates in the active treatment groups.

In the Dacogen arm (integrated AML studies) 37% of patients discontinued treatment due to adverse events.

Post marketing experience

An estimate of cumulative post marketing exposure from launch of DACOGEN to 30 December 2010 was approximately 43,000 treatment courses assuming the 3-Day dosing regimen, an average body surface area of 1.73 m^2, and 3 cycles of treatment (27 3-hour infusions). The post-marketing safety database, which captures spontaneous adverse event reporting, retrieved a total of 448 cases of which 335 were considered serious (74.7%).

2.6.1. Discussion on clinical safety

Overall, the clinical development programme of Dacogen included 1,114 patients who received at least one dose of decitabine, regardless of the disease (AML/MDS), dosage and treatment duration. The clinical safety analysis was mainly based on two studies (Studies DACO-016 and DACO-017) in patients with AML and four studies in patients with MDS (Studies DACO-020, ID03-0180, D-0007, EORTC-06011).

Patients who were included in the AML studies were unfortunately different in term of exposure. Therefore, the two studies could a priori not be pooled and results taken from the provided integrated AML study analysis should be taken with caution. And only data collected in the pivotal study DACO-016 are considered relevant to provide a comprehensive assessment of the safety of DACOGEN in the claimed indication, i.e. the treatment of adult patients with newly diagnosed de novo or secondary AML.

More than 99% of subjects in the DACOGEN and cytarabine treatment groups experienced adverse events. All types of adverse events occurred at lower rates in the TC group.

Myelosuppression and complications of myelosuppression, including infections and bleeding that occur in patients with AML may be exacerbated with Dacogen treatment. In clinical studies, the majority of patients had baseline Grade 3/4 myelosuppression. In patients with baseline Grade 2 abnormalities, worsening of myelosuppression was seen in most patients and more frequently than in patients with baseline Grade 1 or 0 abnormalities. Myelosuppression caused by Dacogen is reversible. Complete blood and platelet counts should be performed regularly, as clinically indicated and prior to each treatment cycle. In the presence of myelosuppression or its complications, treatment with Dacogen may be interrupted or supportive measures instituted.

The percentage of patients experiencing treatment emergent adverse events leading to dose delay or cycle delay was higher in Dacogen group than in cytarabine group. The SmPC section 4.2 highlights that approximately one-third of patients receiving DACOGEN required a dose-delay.

In DACO-016 patients received a median of 10 weeks of cytarabine treatment and 19 weeks of DACOGEN treatment. The exposure-adjusted death rate should be used with caution especially in oncology area. Therefore, the list of adverse drug reactions presented in Section 4.8 of the SmPC highlights those adverse reactions which had a fatal outcome and that in clinical studies, 30% of patients treated with DACOGEN and 25% of patients treated in the comparator arm had adverse events with an outcome of death during treatment or within 30 days of last dose of study drug.

As expected from its mechanism of action interacting with DNA methylation, decitabine is teratogenic in animals, causes impairment on male fertility and is mutagenic. No clinical data are available.

Acute myeloid leukemia is the most common form of acute leukemia in adults. The incidence of AML increases with age and is primarily a disease of older (>60 years) individuals. Over this age pregnancies are not expected but we can easily imagine that younger women could be exposed especially because of the possible use of decitabine to treat severe sickle cell disease.

Having all these elements in mind, the CHMP agrees with the wording of section 4.4, as proposed by the Applicant.

Patients with a history of severe congestive heart failure or clinically unstable cardiac disease were excluded from clinical studies and therefore the safety and efficacy of Dacogen in these patients has not been established.

From the safety database all the adverse reactions reported in clinical trials and post-marketing have been included in the Summary of Product Characteristics.

2.6.2. Conclusions on the clinical safety

Overall, the safety profile of decitabine is globally in accordance with what could be expected from a cytotoxic compound in such a setting. The incidence of serious and/or drug related AEs was slightly higher in the decitabinearm than in the TC/Cytarabine arm. Infections were the most commonly reported drug related adverse event: decitabine -treated subjects had higher rates of most infections than the cytarabine groups, including Grade 3 or 4 infections. Febrile neutropenia, pneumonia, and urinary tract infection were the most common infections.

Percentages of early deaths were comparable between the cytarabine (8.7%) and decitabine (8.5%) treatment groups. Percentages of subjects who died during treatment or within 30 days after the last dose of study drug were also similar: 30.3% in the cytarabine treatment group and 32.8% in the decitabine treatment groups. More deaths were apparently attributed to cardiac causes in decitabine -treated subjects in DACO-016 (13 subjects who received decitabine versus 6 who received cytarabine).

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The applicant submitted a risk management plan.

Table 22: Summary of the risk management plan

Safety concern	**Agreed Pharmacovigilance Activities (routine and additional)**	**Agreed Risk Minimisation Activities (routine and additional)**
Important Identified Risks		
Neutropenia	Routine pharmacovigilance	Myelosuppression (including neutropenia) is a common toxicity associated with the use of DACOGEN (SmPC, Section 4.2). To reduce the risk of myelosuppression

Safety concern	Agreed Pharmacovigilance Activities (routine and additional)	Agreed Risk Minimisation Activities (routine and additional)
		and its complications or to manage myelosuppression should it occur, recommendations include: • to perform complete blood and platelet counts regularly (SmPC, Section 4.4), • to delay or interrupt the dose of DACOGEN in the presence of myelosuppression (SmPC, Sections 4.2 and 4.4), • to initiate supportive treatments (eg, prophylactic antibiotics and/or growth factor support for neutropenia; intravenous anti-infectives and extensive supportive care for active infections) according to institutional guidelines (SmPC, Sections 4.2 and 4.8). No additional risk minimisation activities are proposed.
Anaemia	Routine pharmacovigilance	Myelosuppression (including anaemia) is a common toxicity associated with the use of DACOGEN (SmPC, Section 4.2). To reduce the risk of myelosuppression and its complications or to manage myelosuppression should it occur, recommendations include: • to perform complete blood and platelet counts regularly (SmPC, Section 4.4), • to delay or interrupt the dose of DACOGEN in the presence of myelosuppression (SmPC, Sections 4.2 and 4.4), • to initiate supportive treatments (eg, blood transfusions) according to institutional guidelines (SmPC, Sections 4.2 and 4.8) No additional risk minimisation activities are proposed.
Thrombocytopenia	Routine pharmacovigilance	Myelosuppression (including thrombocytopenia) is a common toxicity associated with the use of DACOGEN (SmPC, Section 4.2). To reduce the risk of myelosuppression and its complications or to manage myelosuppression should it occur, recommendations include: • to perform complete blood and platelet counts regularly (SmPC, Section 4.4), • to delay or interrupt the dose of DACOGEN in the presence of myelosuppression (SmPC, Sections 4.2 and 4.4), • to initiate supportive treatments (eg, blood transfusions) according to institutional guidelines (SmPC, Sections 4.2 and 4.8). No additional risk minimisation activities are proposed.
Pancytopenia	Routine pharmacovigilance	Myelosuppression (including pancytopenia) is a common toxicity associated with the use of DACOGEN (SmPC, Section 4.2). To reduce the risk of myelosuppression and its complications or to manage myelosuppression should it occur, recommendations include: • to perform complete blood and platelet counts regularly (SmPC, Section 4.4), • to delay or interrupt the dose of DACOGEN in the presence of myelosuppression (SmPC, Sections 4.2 and 4.4), • to initiate supportive treatments (eg, prophylactic antibiotics and/or growth factor support for neutropenia; intravenous anti-infectives and extensive supportive care for active infections) according to institutional guidelines (SmPC, Sections 4.2 and 4.8). No additional risk minimisation activities are proposed.
Febrile neutropenia	Routine pharmacovigilance	Febrile neutropenia is a very common adverse event related to myelosuppression in both treated and untreated patients with AML (SmPC, Sections 4.2 and 4.8).

Safety concern	Agreed Pharmacovigilance Activities (routine and additional)	Agreed Risk Minimisation Activities (routine and additional)
		Routine risk minimisation measures for myelosuppression-related febrile neutropenia are those previously described for complications of myelosuppression, ie: • to perform complete blood and platelet counts regularly (SmPC, Section 4.4), • to delay or interrupt the dose of DACOGEN in the presence of myelosuppression or its complications (eg, febrile neutropenia) (SmPC, Sections 4.2 and 4.4), • to initiate supportive treatments (eg, prophylactic antibiotics and/or growth factor support for neutropenia; intravenous anti-infectives or extensive supportive care for active infections) according to institutional guidelines (SmPC, Sections 4.2 and 4.8). No additional risk minimisation activities are proposed.
Pneumonia	Routine pharmacovigilance	Pneumonia is a very common adverse drug reaction in patients receiving DACOGEN (SmPC, Section 4.8). Routine risk minimisation measures for myelosuppression-related pneumonia are those previously described for complications of myelosuppression, ie: • to perform complete blood and platelet counts regularly (SmPC, Section 4.4) • to delay or interrupt the dose of DACOGEN in the presence of myelosuppression or its complications (eg, pneumonia) (SmPC, Sections 4.2 and 4.4), • to initiate supportive treatments (eg, prophylactic antibiotics and/or growth factor support for neutropenia; intravenous anti-infectives or extensive supportive care for active infections) according to institutional guidelines (SmPC, Sections 4.2 and 4.8). No additional risk minimisation activities are proposed.
Sepsis/septic shock	Routine pharmacovigilance	Sepsis/septic shock are common adverse drug reactions in patients receiving DACOGEN (SmPC, Section 4.8). Routine risk minimisation measures for sepsis/septic shock are those previously described for complications of myelosuppression, ie: • to perform complete blood and platelet counts regularly (SmPC, Section 4.4) • to delay or interrupt the dose of DACOGEN in the presence of myelosuppression or its complications (eg, sepsis/septic shock) (SmPC, Sections 4.2 and 4.4), • to initiate supportive treatments (eg, prophylactic antibiotics and/or growth factor support for neutropenia; intravenous anti-infectives and extensive supportive care for active infections, according to institutional guidelines (SmPC, Sections 4.2 and 4.8). No additional risk minimisation activities are proposed.
Haemorrhage	Routine pharmacovigilance	Serious bleeding-related adverse drug reactions in the context of thrombocytopenia are common adverse drug reactions in patients receiving DACOGEN (SmPC, Section 4.8). Routine risk minimisation measures for haemorrhage are those previously described for complications of myelosuppression, ie: • to perform complete blood and platelet counts regularly (SmPC, Section 4.4) • to interrupt or delay the dose of DACOGEN in the presence of myelosuppression or its complications(eg, haemorrhage) (SmPC, Sections 4.2 and 4.4),

Safety concern	Agreed Pharmacovigilance Activities (routine and additional)	Agreed Risk Minimisation Activities (routine and additional)
		• to initiate supportive treatments (eg, transfusions for anaemia and/or thrombocytopenia) according to institutional guidelines (SmPC, Section 4.8). No additional risk minimisation activities are proposed.
Urinary tract infection	Routine pharmacovigilance	Urinary tract infection is a very common adverse drug reaction in patients receiving DACOGEN (SmPC, Section 4.8). Routine risk minimisation measures for other infections are those previously described for complications of myelosuppression, ie: • to perform complete blood and platelet counts regularly (SmPC, Section 4.4) • to delay or interrupt dosing of DACOGEN in the presence of myelosuppression and its complications (eg, other infections) (SmPC, Sections 4.2 and 4.4), • to initiate supportive treatments (eg, prophylactic antibiotics and/or growth factor support for neutropenia; intravenous anti-infectives and extensive supportive care for active infections, according to institutional guidelines (SmPC, Sections 4.2 and 4.8) No additional risk minimisation activities are proposed.
Other Infections (excluding pneumonia and sepsis/septic shock, UTI)	Routine pharmacovigilance	Other infections such as sinusitis are common adverse drug reactions in patients receiving DACOGEN (SmPC, Section 4.8). Routine risk minimisation measures for other infections are those previously described for complications of myelosuppression, ie: • to perform complete blood and platelet counts regularly (SmPC, Section 4.4) • to delay or interrupt dosing of DACOGEN in the presence of myelosuppression and its complications (eg, other infections) (SmPC, Sections 4.2 and 4.4), • to initiate supportive treatments (eg, prophylactic antibiotics and/or growth factor support for neutropenia; intravenous anti-infectives and extensive supportive care for active infections, according to institutional guidelines (SmPC, Sections 4.2 and 4.8) No additional risk minimisation activities are proposed.
Hypersensitivity including anaphylactic reaction	Routine pharmacovigilance	The SmPC (Section 4.3) indicates that decitabine is contraindicated in patients with a hypersensitivity to decitabine or to any of the excipients. Hypersensitivity including anaphylactic reaction is an ADR in Section 4.8 of the SmPC. No additional risk minimisation activities are proposed.
Important Potential Risks		
Reproductive toxicity	Routine pharmacovigilance	Decitabine is teratogenic in rats and mice (SmPC, Section 5.3). Adequate data on the use of DACOGEN in pregnant women are not available (SmPC, Section 4.6). Therefore: • Women of childbearing potential must use effective contraceptive measures. • Men should use effective contraceptive measures and be advised not to father a child while receiving DACOGEN, and for 3 months after completion of treatment • Women who become pregnant should be advised of the potential hazard to the foetus. No additional risk minimisation activities are proposed
Carcinogenicity	Routine pharmacovigilance	The SmPC (Section 5.3) indicates that carcinogenicity studies were not performed using decitabine. Evidence from the literature indicates that decitabine has

Safety concern	Agreed Pharmacovigilance Activities (routine and additional)	Agreed Risk Minimisation Activities (routine and additional)
		carcinogenic potential.
Genotoxicity	Routine pharmacovigilance	The SmPC (Section 5.3) indicates that available data from in vitro and in vivo studies provide sufficient evidence that decitabine has genotoxic potential. No additional risk minimisation activities are proposed The SmPC (Section 4.6) recommends that: • Women of childbearing potential must use effective contraceptive measures • Men should use effective contraceptive measures and be advised not to father a child while receiving DACOGEN, and for 3 months after completion of treatment • Women who become pregnant should be advised of the potential hazard to the foetus. No additional risk minimisation activities are proposed
Important missing information:		
Use in paediatric patients	Routine pharmacovigilance. Safety monitoring of clinical trials included in the PIP: Phase 1/2 trial and a Phase 3 trial in paediatric subjects with AML	The SmPC (Section 4.2) indicates that the safety and efficacy of DACOGEN in children aged <18 years have not yet been established. No additional risk minimisation activities are proposed.
Use in nursing mothers	Routine pharmacovigilance	It is not known whether decitabine or its metabolites are excreted in breast milk. Therefore, the use of DACOGEN is contraindicated during lactation (SmPC, Sections 4.3 and 4.6). No additional risk minimisation activities are proposed
Use in severe renal impairment	Routine pharmacovigilance Targeted follow-up for spontaneous reports through a guided questionnaire	The effect of renal impairment on the pharmacokinetics of decitabine has not been formally studied (SmPC, Section 5.2).The need for dosage adjustment in patients with renal impairment has not been evaluated (SmPC, Sections 4.2). Caution should be exercised, and careful monitoring is advised, in patients with severe renal impairment (CrCl <30 ml/min) (SmPC, Section 4.4). No additional risk minimisation activities are proposed
Use in hepatic impairment	Routine pharmacovigilance Targeted follow-up for spontaneous reports through a guided questionnaire	The effect of hepatic impairment on the pharmacokinetics of decitabine has not been formally studied (SmPC, Section 5.2). DACOGEN should be used with caution in patients with hepatic impairment, and patients should be closely monitored (SmPC, Sections 4.2 and 4.4). No additional risk minimisation activities are proposed
Use in severe cardiac disease (eg, uncontrolled angina or severe CHF [NYHA III-IV])	Routine pharmacovigilance Targeted follow-up for spontaneous reports through a guided questionnaire	The safety of DACOGEN has not been established in patients with a history of severe congestive heart failure or clinically unstable cardiac disease (SmPC, Section 4.4). No additional risk minimisation activities are proposed
Use in non-white populations	Routine pharmacovigilance	The safety of DACOGEN has not been established in non-white populations. Most of the patients studied were white However, the population pharmacokinetic analysis of decitabine indicated that race had no apparent effect on the exposure to decitabine. (SmPC, Section 5.2) No additional risk minimisation activities are proposed
Use in patients under 65 years	Routine pharmacovigilance	DACOGEN is indicated for the treatment of adult patients age 65 years and above (SmPC, Section 4.1). However, the safety profile of DACOGEN is not expected to differ in this population from that in older patients. No additional risk minimisation activities are proposed
Use in AML with CNS involvement	Routine pharmacovigilance	The safety of DACOGEN has not been evaluated in patients with promyelocytic leukaemia or CNS

Safety concern	Agreed Pharmacovigilance Activities (routine and additional)	Agreed Risk Minimisation Activities (routine and additional)
		leukaemia (SmPC, Section 5.1) No additional risk minimisation activities are proposed
Subcutaneous administration and its consequences	Routine pharmacovigilance	The safety of DACOGEN via subcutaneous administration has not been evaluated. DACOGEN is intended for intravenous use under the supervision of experienced physicians. (SmPC, Section 4.2). No additional risk minimisation activities are proposed.
AML = acute myeloid leukaemia, CHF=congestive heart failure, NYHA=New York Heart Association, PIP=Paediatric Investigational Plan, PSUR=Periodic Safety Update Report, SmPC=Summary of Product Characteristics		

No additional risk minimisation activities were required beyond those included in the product information.

2.8. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

3. Benefit-Risk Balance

Benefits

Beneficial effects

Decitabine is a cytidine deoxynucleoside analogue intended for the treatment of adult patients with newly diagnosed de novo or secondary acute myeloid leukaemia. Acute myeloid leukemia is a clonal disorder caused by malignant transformation of a bone marrow-derived, myeloid stem cell or progenitor cell, which demonstrates arrested maturation and aberrant differentiation. Untreated, AML is a rapidly progressing and fatal disease that requires prompt attention.

The management of older patients with AML is guided by performance status, cytogenetics, and the presence of comorbidities which limit the ability of the patient to tolerate cytotoxic (combination) induction chemotherapy. For patients 75 years or older, or those less than 75 for whom intensive chemotherapy is not appropriate, palliative therapy with hydroxyurea or 6-mercaptopurine with supportive care measures are used for white blood cell count control. Several studies suggested that low-dose cytarabine may be effective in this population.

This MAA is mainly based on one single pivotal study, a Phase III, randomized, multicenter study of DACOGEN compared with Treatment Choice (TC) consisting of the subject's choice (with physician's advice) of the current standard-care options of low-dose cytarabine or supportive care. These results are supported by a single-arm, Phase II study, DACO-017, in patients with AML.

The main objective of the pivotal study was to compare DACOGEN, administered at a dose of 20 mg/m2 once daily for 5 consecutive days every 4 weeks, with TC. Overall, 485 subjects were enrolled.

The analysis of the primary endpoint shows an increase in median survival of 2.7 months, which is considered as clinically meaningful in such a particular setting. However, it did not reach statistical

significance (p=0.1079) during the first analysis. Statistical significance was only reached after a second post hoc analysis with methodological concerns (HR 0.82, CI 0.68, 0.99, p=0.0373).

A statistically significant difference was also shown for the secondary endpoint of CR+CRp rate in favour of DACOGEN (17.8%) versus TC (7.8%), p=0.0011 and the median time to response was 4.3 months (95% CI: 3.8, 5.1) and responses were durable (median: 8.3 months, 95% CI: 6.2, 11.4).

The median PFS was 2.1 months in the TC arm and 3.7 months in the DACOGEN arm (HR=0.75; 95% CI: 0.62, 0.91, p=0.0031).

The beneficial effects seen in the pivotal study were also seen in the small uncontrolled phase II study with a reported median EFS of 5.7 months (CI 3.1, 8.1), response rate of 23.6% (CI 13.2, 37) and overall survival of 7.6 months (CI 5.7, 11.5). In addition, patients in the age group between 60 to 64 years were also recruited in this phase II study and showed similar results to patients in older age groups (median OS patients 60-64 years 7.7 months, CI: 3.5, 15.2).

Uncertainty in the knowledge about the beneficial effects.

None.

Risks

Unfavourable effects

More than 99% of subjects in the DACOGEN and cytarabine treatment groups experienced adverse events. All types of adverse events occurred at lower rates in the TC group.

The most frequently observed adverse events in the decitabine group of the AML studies (occurring in ≥ 30% of all subjects) were pyrexia, thrombocytopenia, anaemia, nausea, febrile neutropenia, neutropenia, and diarrhoea. The most frequently observed Grade 3 and 4 adverse events were related to myelosuppression, as expected in AML population. In the AML studies, DACOGEN-treated subjects had higher rates of most infections than the cytarabine groups, including Grade 3 or 4 infections. Febrile neutropenia, pneumonia, and urinary tract infection were the most common infections.

Treatment-emergent serious adverse events were more common in decitabine -treated subjects (80%) than in subjects treated with cytarabine (73%) due to higher rates of Infections and Infestations, Blood and Lymphatic System Disorders, and Respiratory, Thoracic and Mediastinal Disorders. Moreover in the decitabine group, except for “vascular disorders” and “nervous system disorders”, all the percentage of serious adverse events are higher than in cytarabine group.

In the integrated AML studies, the percentages of subjects who died during treatment or within 30 days after the last dose of study drug were 30.3% in the cytarabine treatment group and 32.8% in the decitabine treatment groups. The Applicant states that more deaths were attributed to cardiac causes in decitabine -treated subjects in DACO-016; more than 60% of these cases were from a single country (Poland), with 6 cases from a single site.

Ninety-eight percent of the 530 subjects in the integrated AML studies discontinued treatment, most often due to disease progression in the cytarabine (55%) and decitabine (44%) groups. For the 530 subjects in the integrated AML studies, adverse events led to treatment discontinuation for 10% of subjects in the Supportive Care group, 47% in the cytarabine group, and 37% in the Decitabine group.

The most frequently reported events leading to treatment discontinuation were disease progression (cytarabine: 16%; decitabine: 11%), and general physical health deterioration (cytarabine: 13%; decitabine: 9%). In the Supportive Care group, the most frequently reported adverse event leading to treatment discontinuation was bronchopneumonia (7%; cytarabine: <1%; decitabine: <1%). Drug-related adverse events leading to treatment discontinuation were experienced by 8% of subjects in the cytarabine group and 7% in the decitabinegroup. The majority of these were due to myelosuppressive events or infections. No drug-related adverse event led to treatment discontinuation for more than 1% of subjects in either treatment group.

The overall safety profile of Dacogen was similar to low dose cytarabine and while some undesirable effects were seen more often with Dacogen (such as infections and febrile neutropaenia) it may be due to the longer exposure to Dacogen treatment (median 4 cycles Dacogen versus 2 cycles Cytarabine). The use of supportive anti-infectives and cytokines was also similar in both treatment arms.

Non clinical data have shown irreversible inhibition of spermatogenesis but the relevance of this finding is considered limited in the older AML patient population.

No data is available in patients with severe renal or hepatic impairment but no significant toxicity was seen in relation to the kidneys or liver. There is appropriate caution wording in the SPC for the use of Dacogen in patients with liver impairment and renal impairment.

Uncertainty in the knowledge about the unfavourable effects

The extent of data submitted (682 subjects treated with Dacogen) is satisfactory and no significant uncertainty regarding unfavourable effects has been raised.

Benefit-risk balance

Importance of favourable and unfavourable effects

The analysis of the primary endpoint of the pivotal study shows an increase in median survival of 2.7 months, which is considered as clinically meaningful in such a particular setting. Statistical significance was reached after a second post hoc analysis.

Response rates showed also a highly significant benefit in favour of DACOGEN. Most of all, responses were durable. The difference in median PFS was statistically significant and in favour of the Dacogen arm. Most of these beneficial effects were also observed in the small uncontrolled phase II study.

Given the very poor prognosis in this cancer population an improvement in survival of at least an extra 2.7 months is considered of clinical relevance. Other favourable effects in response rate, progression free survival and event free survival when compared to standard of treatment are also considered to be very important from the clinical point of view.

Overall, the safety profile of decitabineis globally in accordance with what could be expected from a cytotoxic compound in such a setting.

Benefit-risk balance

The benefit-risk balance is considered positive in view of the clinically relevant effect in terms of overall survival and an acceptable safety profile.

Discussion on the benefit-risk balance

Benefits of currently available treatments for AML are diminished with advancing age because of the greater frequency of poor prognostic indicators: an increase in median survival of 2.7 months, as observed in study DACO-016 is therefore considered as clinically meaningful in such a particular setting.

The issue of a possible positive impact of patients who might benefit from induction therapy on study DACO-016 results has been addressed by a post-hoc analysis that only included patients who would not be considered suitable for induction chemotherapy. This analysis, which showed that approximately 94% of patients in DACO-016 would not be considered suitable for induction chemotherapy, was not meaningfully different from the ITT-analysis. As a consequence, it is considered that the use of Dacogen should be limited to to adult patients aged 65 years and above with newly diagnosed *de novo* or secondary AML according to the WHO classification who are not considered candidates for standard induction chemotherapy.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Dacogen is not similar to Ceplene, Trisenox and Vidaza within the meaning of Article 3 of Commission Regulation (EC) No. 847/200.

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Dacogen in the treatment of adult patients age 65 years and above with newly diagnosed de novo or secondary acute myeloid leukaemia (AML), according to the World Health Organisation (WHO) classification, who are not candidates for standard induction chemotherapy, is favourable and therefore recommends the granting of the marketing authorisation.

Conditions or restrictions regarding supply and use

Restricted medical prescription (See Annex I: Summary of Product Characteristics, section 4.2).

Conditions and requirements of the Marketing Authorisation

Risk Management System

The MAH must ensure that the system of pharmacovigilance, presented in Module 1.8.1 of the marketing authorisation, is in place and functioning before and whilst the product is on the market.

The MAH shall perform the pharmacovigilance activities detailed in the Pharmacovigilance Plan, as agreed in version 2.2 of the Risk Management Plan (RMP) presented in Module 1.8.2 of the marketing authorisation and any subsequent updates of the RMP agreed by the CHMP.

As per the CHMP Guideline on Risk Management Systems for medicinal products for human use, the updated RMP should be submitted at the same time as the next Periodic Safety Update Report (PSUR).

In addition, an updated RMP should be submitted:

- When new information is received that may impact on the current Safety Specification, Pharmacovigilance Plan or risk minimisation activities
- Within 60 days of an important (pharmacovigilance or risk minimisation) milestone being reached
- at the request of the EMA

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Not applicable

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

New Active Substance Status

Based on the CHMP review of data on the quality of the active substance, the CHMP considers that decitabine is qualified as a new active substance.

Paediatric Data

Furthermore, the CHMP reviewed the available data of studies subject to the agreed Paediatric Investigation Plan P/52/2011 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.