CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:NDA 21041

PHARMACOLOGY REVIEW(S)

Division of Oncology Drug Products, HFD-150
Review and Evaluation of Pharmacology and Toxicology Data
Original Review

Keywords: Liposomal, reproductive toxicity, genetic toxicity, cytarabine

NDA: 21-041
Serial #:000
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Reviewer: Doo Y. Lee Ham, Ph. D.

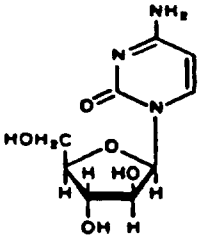
Date Review Completed: March 24, 1999

Applicant: DepoTech Corporation
San Diego, CA 92121

Drug Name: Primary: DepoCyt™ Encapsulated Cytarabine (DTC-101); Lipo-C
USAN: Cytarabine (Ara-C)
Other Names: Cytosine Arabinoside; Aracytidine; Aracytine; Ara-C; Cytosar
Code Name: U-19920

Chemical Name: 4-Amino-1-b-D-arabinofuranosyl-2(1H)-pyrimidinone; 1-b-D-arabinofuranosylcytosine; b-cytosine arabinoside

Structure:



Molecular Formula: $C_9H_{13}N_3O_5$

Molecular Weight: 243.22

Related IND & NDA: IND
NDA 16-793, Cytarabine (Upjohn)

Class: Antimetabolite/Antineoplastic agent

Indication: DepoFoam™ Encapsulated Cytarabine (DTC 101) is intended for intrathecal administration in the treatment of meningeal metastases.

Proposed dose: 50 mg cytarabine (~29 mg/m2)

Route of Administration: Intrathecal administration

Previous Reviews, Dates and Reviewers:

IND				4/2/87	CNeilsen
Review #2	"	"	"	11/22/89	WCoulter
NDA	"	"	"	3/17/98	DYLeeHam

Studies Reviewed Previous Submission:

I. Pharmacology:
- A. Preclinical Efficacy and Pharmacokinetics study of Subcutaneously Administered Cytarabine Entrapped in Multivesicular Liposomes, Using a Mouse Tumor Model Study No. DTC-P001)
- B. Preclinical Efficacy and Pharmacokinetics Studies in Mice Following intraperitoneal Administration of Multivesicular Liposomes Containing Cytarabine (Entrapped in the Presence or Absence of Hydrochloric Acid), with and without Treatment With "Blank" Liposomes (Study No. DTC-P002/3)

II. Pharmacokinetics
- A. Pharmacokinetics Study of Multivesicular Liposomes Containing Cytosine Arabinoside After Intrathecal Administration in Rats (Study No. DTC-P004)
- B. Pharmacokinetics Study of DepoFoam Encapsulated Cytarabine (DTC 101) After Intrathecal Injection in Rhesus Monkeys (Study No. DTC-P006)
- C Distribution of Radiolabeled Lipid and Drug After Intrathecal Administration of DepoFoam™ Encapsulated Cytarabine in the Rat (Report No.033-00008.001)

III. Toxicology
- A. DTC 101 (DepoFoam™ Encapsulated Cytarabine) Toxicokinetic and Range-Finding Intrathecal Injection Study in Monkey (HE Study No. 1363-001)
- B. DTC 101 (DepoFoam™ Encapsulated Cytarabine) 4-Cycle Intrathecal Subchronic Toxicity with recovery period in Rhesus Monkey (HE Study No. 1363-002)
- C DTC 101 (DepoFoam™ Encapsulated Cytarabine) Toxicokinetic Intrathecal Injection Study in Rhesus Monkey (HE Study No. 1363-003)

Studies Reviewed with This Submission:

I. Reproductive Studies: Published data
- A. Ortega, A et al, Maternal and developmental toxicity of low doses of cytosine arabinoside in mice. Teratology 44:379-384, 1991
- B. Percy, DH, Teratogenic effects of the pyrimidine analogues 5-iododeoxyuridine and cytosine arabinoside in late fetal mice and rats. Teratology 11:103-117, 1975
- C. Adlard, BP et al, A Comparison of the effects of cytosine arabinoside and adenine arabinoside on some aspects of brain growth and development in the rat. Brit J Pharmacol 54:33-39, 1975
- D. Chaube, S et al, The teratogenic effect of 1-β-D-arabinofuranosylcytosine in the rat. Biochem Pharmacol 17:1213-1216, 1968

II. Mutagenicity Studies: Published data

A. Kihlman, BA et al, The effect of deoxyadenosine and cytosine arabinoside on the chromosomes of human leukocytes in vitro. Hereditas 50:139-143, 1963

B. Kouri, RE et al, 1-beta-D-arabinofuranosylcytosine-induced malignant transformation of hamster and rat cells in culture. Cancer Res : 35 (9)2413-2419, 1975

C. Hayashi, M et al, Micronucleus test with 1-b-D-arabinofuranosylcytosine administered by intraperitoneal injection and oral gavage. Mutation Res 223:345-348, 1989

D. Beaula, H and Subramanyam, S, Genotoxic evaluation of Ara-C by multiple parameters. Mutation Res 263:185-196, 1991

III. Carcinogenicity Study: Published data

A. Berger, MR and D. Schmahl Study on the Long-term toxic efficacy of cytosine arabinoside in Sprague-Dawley (SD) rats: Cancer Letters 43:59-64, 1988

B. Weisburger, EK (1977) Bioassay Program for Caricinogenic Hazards of Cancer Chemotherapy. Cancer 40:1935-77

Studies not Reviewed: Published data

I. Reproductive Study:

A. Gough, AW et al, Comparison of the neonatal toxicity of two antiviral agents: Vidarabine phosphate and cytarabine. Toxicol Appl Pharmacol 66:143-152, 1982

B. Karnofsky, DA and CR Lacon, The effect of 1-β-D-arabinofuranosylcytosine on the development chick embryo. Biochem Pharmacol 15:1435-442, 1966

C. Shimada, M et al, Cytarabine and its effect on cerebellum of suckling mouse. Arch Neurol 32:555-559, 1975

II. Mutagenic Study:

A. Nichols, WW and Heneen, WK, Chromosomal effects of arabinosylcytosine in a human deploid cell strain. Hereditas 52:402-410, 1965

B. Scott, WJ et al, Studies on induction of polydactyly in rats with cytosine arabinoside. Dev Biol 45:103-111, 1975

C. Wobus, AM, Clastogenic activity of cytosine arabinoside and 3'-deoxy-3'-fluorothymidine in Ehrlich ascites tumor cells in vitro. Mutation Res 40:101-106, 1976

Portions of this review were excerpted directly from the sponsor's submission

Background Data:

Cytosar-U^R (sterile Cytarabine for intravenous, intrathecal and subcutaneous administration) the active ingredient in DTC 101, was approved and clinically available to treat for both leukemia and solid tumors since 1969. The primary use for cytarabine is AML in combination with other chemotherapeutic agents. It also is used in the blastic crisis phase of CML, as secondary treatment of ALL, and non-Hodgkins lymphomas. Intrathecal Cytarabine (Ara-C) has been extensively used for the treatment of meningeal metastases from malignant brain tumors and systemic tumors.

DTC 101 (DepoCyt, liposomal C) is cytarabine encapsulated into DepoFoam (multivesicular lipid-based particles) for sustained release. DTC 101 was designed to increase the efficacy of cytarabine while reducing its dose-limiting toxicities by altering the pharmacokinetics (CSF, plasma) and tissue distribution in neoplastic meningitis.

DepoTech has submitted toxicology and pharmacokinetic studies for DTC 101 in primates and published data for the support for safety and efficacy use in intrathecal administration of the drug.

NDA DepoCyt (DTC 101, liposomal cytarabine) for intrathecal treatment of carcinomatous meningitis, was not approved. After the 1997 ODAC meeting, DepoTech submitted additional information to support an application for accelerated approval for the intrathecal treatment of

lymphomatous meningitis under NDA 21-041 on October 15, 1998. The experience with DepoCyt in carcinomatous meningitis was considered as part of the safety database for NDA 21-041 and might provide some additional support for intrathecal anticancer activity.

I. Reproductive Studies: Published data

A. Ortega, A et al, Maternal and developmental toxicity of low doses of cytosine arabinoside in mice. Teratology 44:379-384, 1991

Method:

Pregnant Swiss mice were randomly divided into 4 groups (n=15 or n=13) and given i.p. doses of 0, 0.5, 2, or 8 mg/kg cytarabine on days 6-15 of gestation. Maternal weight, and food consumption were measured pre-, during, and post-treatment period, and clinical signs were observed daily. On day 18, dams were sacrificed and uterine contents were examined. Uterine horns were examined for number of implantation sites, number of resorptions, and dead and live fetuses. All live fetuses were evaluated for body weight, external, visceral and skeletal abnormalities.

Results:

Maternal body weight gain was significantly reduced in dams receiving 2 mg/kg/day (63%) or 8 mg/kg/day (mkd) (70%) on days 12-15. Maternal body weight gain was comparable between treated and control animals during the pretreatment interval (days 0-6), however, during the post-treatment period (days 15-18), the weight gain in the 8 mkd was significantly lower (88%) than the control group. Maternal food consumption was significantly reduced on gestational days 6-15 at both doses.

As in Table 2, a number of implantations was similar in all groups, however, the i.p. doses of Ara-C at 8 mkd significantly increased the number of early and late resorptions and significantly decreased the number of live fetuses. Fetal body weight was significantly reduced in all treated groups, and the number of stunted fetuses was significantly increased at 8 mkd.

TABLE 2. Summary of observations at time of cesarean sections of mice treated with Ara-C[a]

	Dose level (mg/kg/day)			
	0	0.5	2	8
Number of pregnant animals	+15	+15	+15	+13
Dam weight	49.7 ± 6.2	49.2 ± 8.0	45.5 ± 7.6	37.6 ± 2.7***
Gravid uterine weight	14.6 ± 5.3	15.1 ± 6.9	12.2 ± 4.8	2.2 ± 1.7***
Corrected body weight change[b]	6.2 ± 2.2	5.2 ± 2.8	4.4 ± 2.6	4.8 ± 2.3
Implantations/litter	9.7 ± 3.4	10.7 ± 5.3	8.9 ± 3.4	8.6 ± 4.0
Early resorptions/litter	0.9 ± 0.6	0.5 ± 0.8	0.8 ± 0.9	4.9 ± 3.8***
Late resorptions/litter	0.2 ± 0.4	0.2 ± 0.4	0.0 ± 0.0	2.8 ± 3.2*
Dead fetuses/litter	0.0 ± 0.0	0.1 ± 0.3	0.1 ± 0.4	0.2 ± 0.6
Live fetuses/litter	8.6 ± 3.3	9.9 ± 5.0	8.0 ± 3.4	0.9 ± 1.9***
Fetal body weight	1.36 ± 0.12	1.21 ± 0.17**	1.20 ± 0.16**	0.67 ± 0.19**
Number of stunted fetuses	2 (2)	4 (2)	6 (3)	10 (3)*

[a]Values are means ± SD. Numbers in parentheses indicate the number of affected litters.
[b]Corrected body weight change = body weight gain during gestation − gravid uterine weight.
*Significantly different from the control value, $P < 0.05$.
**Significantly different from the control value, $P < 0.01$.
***Significantly different from the control value, $P < 0.001$.

The type and frequency of external and visceral malformations are summarized in Table 3 and 4.

TABLE 3. Incidence of external and visceral anomalies in mice treated with Ara-C on days 6 to 15 of gestation[1]

	Dose level (mg/kg/day)			
	0	0.5	2	8
Number of fetuses examined externally	129 (15)	149 (15)	140 (15)	11 (4)
Phocomelia of forelimbs and hindlimbs	0 (0)	0 (0)	0 (0)	11 (4)***
Oligodactyly in forelimbs	0 (0)	0 (0)	4 (2)	—
Oligodactyly in hindlimbs	0 (0)	0 (0)	2 (2)	—
Polydactyly in hindlimbs	0 (0)	0 (0)	6 (3)	—
Short or absent tail	0 (0)	0 (0)	0 (0)	11 (4)***
Micrognathia	0 (0)	0 (0)	0 (0)	4 (2)*
Total external defects	0 (0)	0 (0)	10 (4)	11 (4)***
Number of fetuses examined viscerally	48 (15)	54 (15)	49 (15)	4 (2)
Cleft palate	0 (0)	1 (1)	7 (4)	3 (2)***
Dilation of cerebral ventricles	0 (0)	0 (0)[2]	2 (2)	2 (2)***
Renoureteral alterations[3]	0 (0)	0 (0)	3 (3)	2 (1)
Total internal soft tissue defects	0 (0)	1 (1)	12 (8)***	3 (2)***

[1]Numbers in parentheses indicate the number of litters.
[2]Agenesis, hypoplasia, and renal ectopia.
*Significantly different from the control value, $P < 0.05$.
**Significantly different from the control value, $P < 0.01$.
***Significantly different from the control value, $P < 0.001$.

TABLE 4. Summary of skeletal examinations in mice treated with Ara-C on days 6 to 15 of gestation[1]

	Dose level (mg/kg/day)		
	0	0.5	2
Number of litters examined	15	15	15
Number of ossified sacrococcygeal vertebrae[2]	6.3 ± 1.29	4.9 ± 1.25*	4.9 ± 1.87*
Number of ossified forelimb proximal phalanges[2]	4.5 ± 0.63	4.2 ± 1.00	3.5 ± 1.37*
Number of ossified forelimb medial phalanges[2]	2.1 ± 1.67	1.3 ± 1.04	0.8 ± 1.21*
Number of ossified hindlimb proximal phalanges[2]	4.3 ± 1.14	3.9 ± 1.37	2.9 ± 1.82*
Number of ossified hindlimb medial phalanges[2]	1.1 ± 1.55	0.0 ± 0.00	0.3 ± 0.58
Fetuses with ossified calcaneus (%)[2]	67 ± 37	27 ± 33*	4 ± 13***
Occipital bone, reduced ossification[3]	3 (2)	2 (2)	9 (3)
Parietal bones, reduced ossification[3]	3 (2)	2 (2)	9 (3)
Asymmetrical sternebrae ossification[3]	4 (4)	5 (5)	7 (5)
Other alterations[3,4]	0 (0)	5 (3)	5 (5)*
Total skeletal defects	8 (5)	13 (10)	15 (10)

[1]The malformations at 8 mg/kg/day were qualitatively different from those presented in this table (see Results section).
[2]Values are means ± SD.
[3]Number of affected fetuses (number of affected litters).
[4]Including extra sternebrae, delayed fusion of mandibles, delayed ossification of mandible and calvaria, and dome-shaped calvaria.
*Significantly different from the control value, $P < 0.05$.
**Significantly different from the control value, $P < 0.01$.
***Significantly different from the control value, $P < 0.001$.

Administration of Ara-C during organogenesis produced maternal and developmental toxicity in mice at all dose levels, but not maternal toxicity in terms of body weight at 0.5 mkd. Ara-C produced a significant increase in skeletal abnormalities at 0.5 and 2 mkd. Fetotoxicity was indicated by reduced fetal weight (≥0.5 mg/kg) and decreases in the number of live fetuses and increased early and late resorptions (8 mg/kg). Increased incidence of visceral malformations was observed at ≥ 2.0 mg Ara-C/kg/day.

B. Percy, DH, Teratogenic effects of the pyrimidine analogues 5-iododeoxyuridine and cytosine arabinoside in late fetal mice and rats. Teratology 11:103-117, 1975

Method:

Pregnant ICR Swiss mice and SD rats were treated for 3 days with 5-iododeoxyuridine (IUDR) or cytosine arabinoside (CA) beginning days of 16 or 18 of pregnancy for mice and rats, respectively. Mice were treated with IUDR at 100, 200 or 400 mg/kg/day and rats were given IUDR at 200 and 400 mg/kg/day and 12.5, 25, 50 mg/kg/day CA were given to mice and rats. All treatments were made sc route, except the administration of IUDR to rats was oral due to its large volume. Twenty-five percent of the animals exposed to each dosage were killed at 10 days and the remainder at 20 days. Mortality and histopathology were evaluated. A midline sagittal section of eye and brain were taken, and longitudinal and transverse sections were taken through the kidney for histological examinations.

Results:

Mortality:

Dose-related mortality occurred in newborn rats. Only 75% and 30% of the newborn rats survived one week in the 25 mkd and 50 mkd groups, respectively. In contrast >96% of the newborn mice survived these doses.

Histopathology:

CNS: In 10- and 20 day-exposed animals with 50 mkd CA showed impaired cerebellum development. The affected areas of cerebellar cortex were reduced in size, the external granular layer varied thickness and was irregular. Purkinje cells were scattered in the internal granular layers, and clear, vacuolated spaces (15 um in diameter) were scattered in the cortex and medulla. These spaces (vacuolated areas) were interpreted to be the defective cerebellar development and alignment of neuronal and glial components in mice. In rats treated 50 mkd, histopathologic findings were similar to those found in mice.

Eye: No abnormalities were found in mice. However, rats treated with 50 mkd showed marked retinal dysplasia in all treated animals.

Kidney: Scattered foci in the superficial renal cortex and aggregations of poorly differentiated cells were present. These cells were interpreted to be precursors to tubular and glomerular structures indicative of defective nephrogenesis in mice. In rats, marked focal microcystic renal change at 50 mkd, and minimal to moderate lesions were observed by lower doses. Additionally, isolated dilated tubules scattered in the renal cortex consisted of a large vacuolated epithelial cells.

C. Adlard, BP et al, A Comparison of the effects of cytosine arabinoside and adenine arabinoside on some aspects of brain growth and development in the rat. Brit J Pharmacol 54:33-39, 1975

Method:

For prenatal treatment, pregnant rats (Lister black and white hooded strain) were treated with i.p. dose of 50 mg/kg Ara-C on day 14 of gestation. All litters were born at either 21 or 22 days of gestation. Up to six rats (2 males, 4 females) were killed at 25 days of age. From each control or Ara-C litters, 2 males were weaned at 25 days and tested for T-maze learning ability when 15 weeks old.

For postnatal treatment, litters were reduced to 6 males at birth. At 5 days of postnatal age 2 rats in each of 6 litters were injected with saline and remaining 4 rats were injected either ara-C 50 mg/kg or Ara-C 250 mg/kg. All animals were killed at 25 days old.

Results:

In prenatal growth and prenatal treatment, a single dose of Ara-C at 50 mg/kg at 14 days of gestation resulted in a reduced litter weight (~14%) and the brain weight (~17%) at birth. The brain/body ratio was significantly reduced as in Table 1. Adult 15 week-old male rats whose mothers were treated with Ara-C (50 mg/kg) prenatally showed specific microcephaly in that body weight was normal but brain weight was reduced by 15% as in Table 6.

Table 1 Effect of single intraperitoneal dose of cytosine arabinoside (ara-C) on day 14 of gestation on mean body and brain weight at birth.

	Control	Ara-C (50 mg/kg)
Body wt. (g)	5.76 ± 0.79 (70)	4.58 ± 0.45* (60)
Brain wt. (mg)	228 ± 24 (31)	189 ± 20* (14)
Brain wt./body wt. ratio (%)	4.21 ± 0.26 (31)	3.82 ± 0.31* (14)

Results (mean ± s.d. of the number of animals indicated in parentheses) are based on the following numbers of litters: control, 7; ara-C (50 mg/kg)

* $P < 0.001$, compared with controls.

Table 3 Effect of a single intraperitoneal dose of cytosine arabinoside (ara-C) on 5 days of postnatal age on mean body and brain weight at 25 days of age.

	Control	Ara-C (50 mg/kg)	Ara-C (250 mg/kg)
Number of rats	11	6	7
Body wt. (g)	55.2 ± 10.1	53.3 ± 14.4	48.3 ± 10.4
Brain wt. (mg)	1387 ± 110	1370 ± 106	1280 ± 111
"Forebrain" + "Stem" (mg)	1188 ± 86	1192 ± 88	1128 ± 85
Cerebellum wt. (mg)	196 ± 24	178 ± 15	152 ± 26
Cerebellum wt./whole brain wt. ratio (%)	14.0 ± 1.0	13.0 ± 0.5	11.8 ± 1.1

Results (mean ± s.d.) are based on 6 litters.
Linear regression analysis of effects of ara-C within this dose range on cerebellum weight and cerebellum weight/whole brain weight ratio shows that both are highly significant ($P < 0.001$).

Table 6 Effect of a single intraperitoneal dose of cytosine arabinoside (ara-C) on day 14 of gestation on mean body and brain weights and on T-maze errors by 15-week-old male rats.

	Control	Ara-C (50 mg/kg)
Number of rats	12	11
Body wt. (g)	304 ± 25	294 ± 32
Brain wt. (mg)	1760 ± 56	1497 ± 68*
Brain wt./body wt. ratio (%)	0.584 ± 0.062	0.512 ± 0.045†
Total T-maze errors (days 1-4)	6.1 ± 3.6	10.9 ± 3.9†

Results (mean ± s.d.) are based on 6 litters of each group.
* $P < 0.001$, † $P < 0.01$, compared with controls.

In postnatal treatment, a single dose of Ara-C at 5 days of age significantly reduced cerebellar weight and the ratio between cerebellar weight and brain weight at 25 days as in Table 3. Following the high dose of 250 mg/kg Ara-C, whole brain weight (1280 mg) was less than that in control (1387 mg), however, there was no effect on body growth in gram (55.2 VS 48.3), respectively.

In T-maze test, during the first four days of testing the control rats improved in both errors made and number of errors runs. In contrast, animals treated prenatally with Ara-C did not improve after day 2. Ara-C rats made significantly more errors than controls on days 3 and 4, and a fewer errorless runs on day 4. Over days 1 to 4, the Ara-C group made 80% more total error than the control in the T-maze test.

Ara-C exerted a much more severe effect on the brain growth when given prenatally, at the time of initiation of rapid neuronal multiplication. Similarly glial cell multiplication is almost exclusively postnatal in the rat and it is this process which must have been inhibited after ara-C treatment at 5 postnatal days of age. This had no effect on whole brain growth which could not be accounted for by the effect on the cerebellum.

The effects of prenatal ara-C in many ways resembled those observed after prenatal treatment with hydroxyurea. Neither ara-C nor hydroxyurea produced any major malformations, but both treatments reduced extent of hair pigmentation and/or migration. Ara-C seemed to produce a more severe effect on the brain than hydroxyurea in that microcephaly (low brain/body ratio) was observed as early as the time of birth.

D. Chaube, S et al, The teratogenic effect of 1-β-D-arabinofuranosylcytosine in the rat. Biochem Pharmacol 17:1213-1216, 1968

Method:

Pregnant Wistar rats were treated with single i.p. doses ranging from 2.5 to 900 mg/kg Ara-C on days 5-12 of gestation. On day 21, all animals were sacrificed and uterine contents were examined. All live fetuses were evaluated for body weight, external, visceral and skeletal malformations.

Results:

The effects of ara-C on the rat fetus are shown in Figure 1A-D. The lowest dose that killed all fetuses by day 21 of gestation was 50 mg/kg on day 9 (Figure 1A). The lowest dose of ara-C that produced malformations was 20 mg/kg (day 11, or 12, Figure 1C, D) and the highest was 800 mg/kg (day 12, Figure 1D).

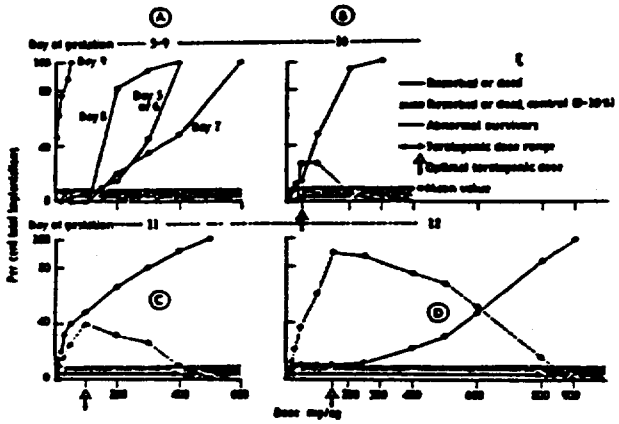


FIG. 1. Lethal and teratogenic effects produced by single i.p. injections of ara-C into the pregnant rat from day 5–12 of gestation; sacrificed on day 21.

The incidence of specific malformations in rats treated with ara-C are summarized in Table 1.

TABLE 1. TERATOGENIC EFFECTS OF ARA-C IN THE RAT FETUS (SINGLE I.P. INJECTIONS INTO THE PREGNANT RAT FROM DAY 10–12 OF GESTATION; SACRIFICED ON DAY 21)

Fetal effects	Day of gestation															
	10			11						12						
Teratogenic dose (mg/kg)	20	100	200	20	50	100	200	300	400	20	50	100	150	250	400–600	800
Fetal mortality (%)	13	48	94	25	39	47	70	76	91	N*	N	N	N	12	22–32	83
Total survivors	45	27	2	39	22	20	8	14	3	66	22	80	25	20	39	2
No. abnormal	14	13	3	5	9	22	8	14	3	8	24	47	25	20	39	2
Numbers with specific abnormalities																
Encephalocele†			1						2							
Cleft palate	6	14	3			11	8	13	3		2	24	23	26	39	2
Cleft lip	4	10	2			4	6	13	3							
Recorded‡ and/or clubbed																
Forelimbs			1			1	8	13	3		1	20	20	26	39	2
Rear legs			1			13	8	13	3	3	10	25	29	26	39	2
Ecro-, cyn-, poly- or brachydactylous																
Forepaw	1							1	3	4	11	22	29	26	39	2
Rear Paw			1	3 - 9		16	4	14	3		16	32	26	20	39	2
Tail, short, kinky or absent	6	6	3			4	4	14	3	3	5	18	13	26	39	2
Micrognathia or agnathia			1			9	8	13	3			7	6	26	39	2

* N = 1–10% (Control value).
† Herniol protrusion of the brain substance through a cleft in the skull.
‡ Micrognathia and incomplete ossification.

Malformations included cleft palate, micrognathia, deformed rear appendages, paws and tail. Skeletal defects included incomplete ossification, distortion and fusion of the bones of the skull and appendages. Other defects included fused ribs and vertebrae and incomplete ossified sternebrae.

II. Mutagenicity Studies:

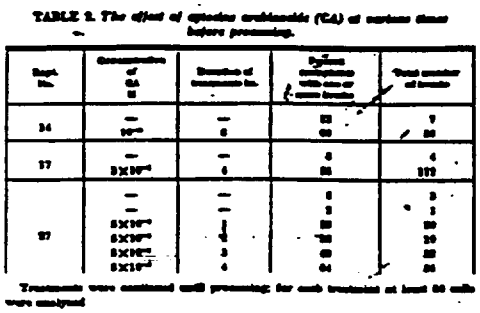
A. Kihlman, BA et al, The effect of deoxyadenosine and cytosine arabinoside qn the chromosomes of human leukocytes in vitro. Hereditas 50:139-143, 1963

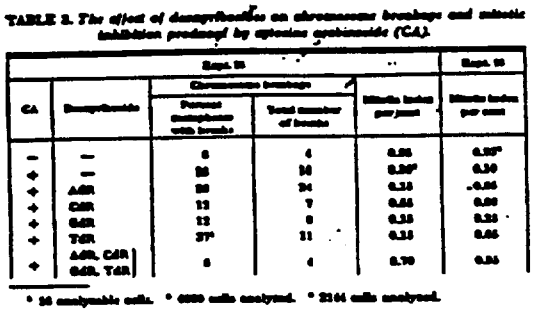
Method:

Human leukocytes (2 or 3 x 10^6 cells/mL) were cultured in culture medium. Cultures were dispersed in 1.5 mL in loosely stopped test tubes and grown at 37°C in 5% CO_2 atmosphere. Cells were exposed to various concentrations (10^{-4}-10^{-6}M) of ara-C. In another set of experiments, cells were exposed to both ara-C and deoxyribosides (AdR, CdR, GdR, or TdR). Cells were processed after one and one-half hrs exposure to colchicine. The cell preparations were fixed and squash preparations were made on siliconized slides.

Results:

Ara-C induced chromosomes aberrations in human leukocytes and the effect consists of gaps and open breaks. Breaks are more frequent towards chromosome ends, often with small terminal fragments. As in the Table 2, ara-C is highly effective when added 3 to 4 hrs before processing and that a definite effect can be obtained as late as one hour before processing.





The mitotic inhibition caused by ara-C is completely or almost completely reversed when all four deoxyribosides are added to the treatment solution. When applied alone, only CdR is effective in reversing the mitotic inhibition produced by ara-C. The chromosome breaking effect of ara-C appears to be completely reversed by the mixture of the four deoxyribosides. AdR and TdR have no influence on the ara-C effect when applied separately.

B. Kouri, RE et al., 1-beta-D-arabinofuranosylcytosine-induced malignant transformation of hamster and rat cells in culture. Cancer Res : 35 (9)2413-2419, 1975

Method:

In vitro transformation, HF (secondary Syrian Golden hamster fetal) and H43 (rat) cell systems were used. Exponentially growing H43 cells were exposed to cytarabine 10^{-5} M for 2 to 24 hrs. Cell transformation was identified visually as focal loss of contact inhibition of cells in culture.

Results:

Transformation was induced by various doses (10^{-3} to 10^{-7}M) of ara-C. A comparison with the transformation effects of BP is also given. Results demonstrate that for ara-C transformed HF cells as little as 6 hr exposure to ara-C was needed for transformation, and the maximum transformation occurred concentrations of 10^{-5} or 10^{-4} M ara-C for 12 to 24 hr as in Table 3.

Table 3

Summary of ara-C-induced transformation in HF cells in vitro

	Colonies transformed[a] after treatment for							
	2 hr		6 hr		12 hr		24 hr	
ara-C (M)								
0	1/361	(0.3)[b]	1/480	(0.2)	1/172	(0.6)	3/582	(0.5)
10^{-5}	2/287	(0.7)	2/316	(0.6)	3/169	(2.6)	7/290	(2.5)
10^{-4}	0/200	(0.0)	5/510	(0.9)	6/174	(3.4)	7/250	(2.8)
10^{-3}	0/222	(0.0)	7/367	(1.9)	7/160	(4.1)	11/238	(4.6)
10^{-2}	0/138	(0.0)	4/200	(2.0)	8/122	(6.5)	8/154	(5.2)
10^{-1}	1/231	(0.4)	7/177	(3.9)	3/98	(3.1)	7/107	(3.7)
BP (M)								
1.2×10^{-5}	0/200	(0.0)	0/280	(0.0)	3/420	(0.7)	10/380	(2.6)
1.2×10^{-4}	1/400	(0.2)	1/420	(0.2)	1/280	(0.3)	3/290	(1.0)

[a] Values given in terms of the number of colonies phenotypically transformed per total number of colonies analyzed. Data represent the summation of 3 separate experiments.
[b] Numbers in parentheses, percentages of transformations.

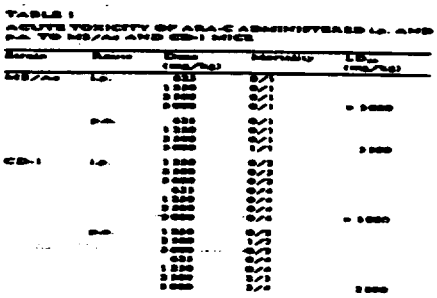
C. Hayashi, M et al, Micronucleus test with 1-b-D-arabinofuranosylcytosine administered by intraperitoneal injection and oral gavage. Mutation Res 223:345-348, 1989

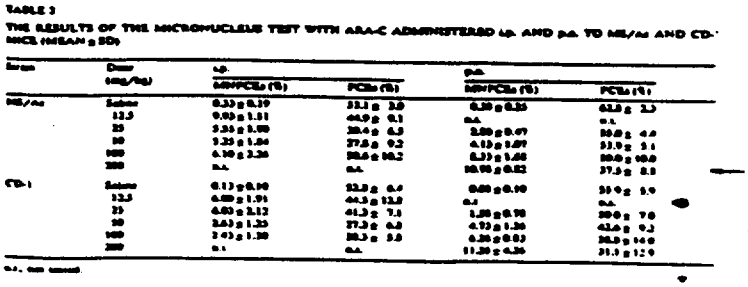
Method:

A pilot acute toxicity test was performed to determine the dose range to be used in the micronucleus test. Male MS/Ae and CD-1 mice (4/group) were given ara-C at 12.5, 25, 50 and 100 mg/kg, i.p., and 25, 50, 100, and 200 mg/kg, p.o. Mice were killed 24 hr after a single administration. The effect of ara-C on the induction of micronuclei was studied using the same i.p. and p.o. doses as in the pilot acute toxicity study. Bone marrow was collected for micronucleus testing. Scoring of micronuclei in mouse bone marrow polychromatic erythrocytes (PCE%) were made to evaluate a clastogenic effect.

Results:

The results of the acute toxicity test are summarized in Table 1. Ara-C has a low acute toxicity: the LD50 values were >5000 mg/kg, i.p. and 3500 mg /kg, p.o. in MS/Ae mice, and >5000 mg/kg, i.p. and 2800 mg/kg, p.o. in CD-1 mice.





The results of the micronucleus test are summarized in Table 2. In MS/Ae mice, low frequencies of MNPCEs were observed in the higher dose groups with i.p. treatment. P. O. administration resulted in a dose-dependent increase in MNPCEs with a peak of ~10% at a dose of 200 mg/kg. A similar pattern of different dose responses for i.p. and p.o. administration was observed in CD-1 mice. After i.p. injection, higher frequencies of MNPCEs were observed at 12.5 mg/kg and 25 mg/kg than at 50 and 100 mg/kg. In p.o. treatment, the frequency of MNPCEs increased dose-dependently and peaked at the highest dose.

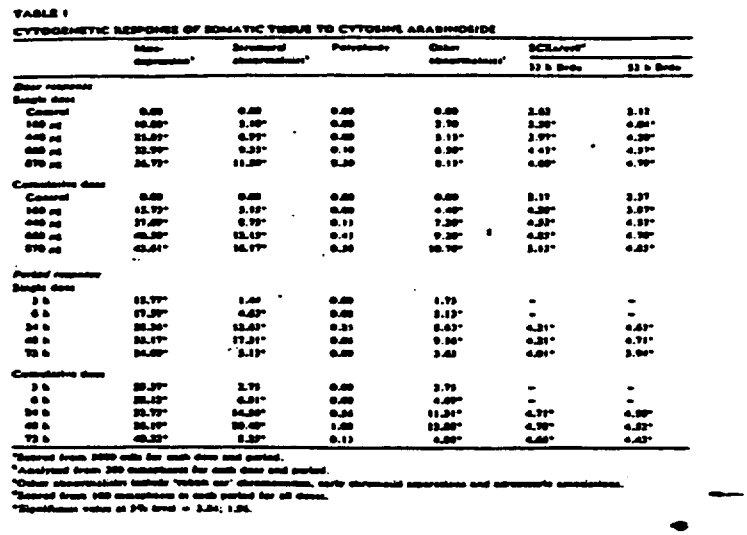
D. Beaula, H and Subramanyam, S, Genotoxic evaluation of Ara-C by multiple parameters. Mutation Res 263:185-196, 1991

Method:

The cytogenetic effects of the ara-C are evaluated using in vivo and in vitro test systems assessing multiple parameters. In in vivo studies, 4 groups of inbred Swiss albino male rats received i.p. doses of 160, 440, 660 and 870 ug ara-C and the 5th group received distilled water as vehicle control. Animals were sacrificed at 3, 6, 24, 48 or 72 hr after single dose or the last day of treatment following daily x 5 administration. Somatic chromosome preparations were made from bone marrow, stained with buffered Giemsa after coding and analyzed for chromosome anomalies. The in vivo sister-chromatid exchange assay was carried out for 24, 48, and 72 hr following single- and multiple doses. In meiotic study, chromosome preparations were made at weekly intervals for 5 weeks to evaluate the action of ara-C on different stages of spermatogenetic cycle and sperm-head abnormality test was carried out for the same period. In in vitro studies, cytogenetic effects on human leukocyte cultures and SCE studies were carried out by adding 12 ug/ml of 5-bromodeoxyuridine to the culture at the time of initiation.

Results:

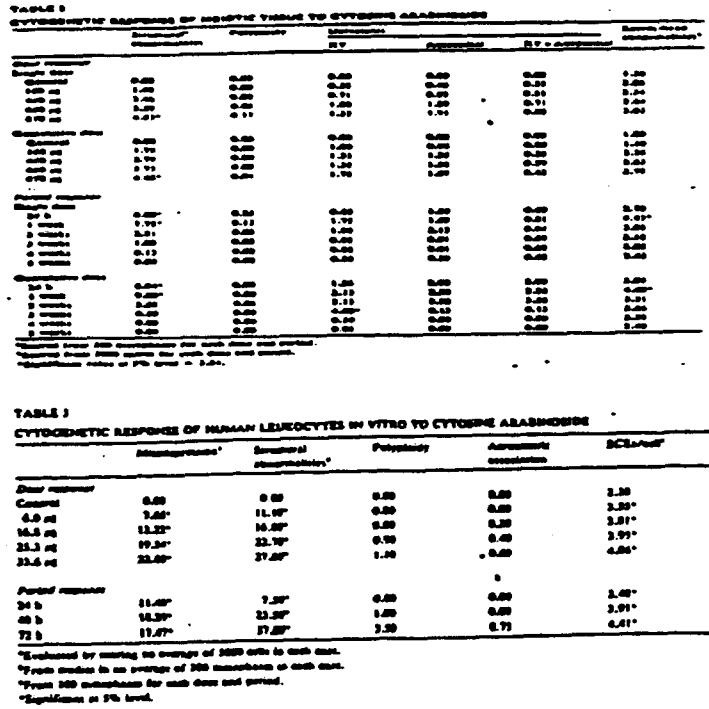
In the vivo somatic system, ara-C exhibited a strong mitodepressive effect in mouse bone marrow cells. The effect was more intense in the multiple doses than the single dose treatment. All periods (3-72 hr) revealed a dose-dependent and statistically significant increase in mitodepression at 72 hr exposure with both series of ara-C as in Table 1. Ara-C caused a dose-dependent increase in structural abnormalities and polyploidy after both single and daily x 5 doses.



A statistically significant dose-dependent increase in SCEs was seen in both series and there was a tendency for gradual decline in the frequencies of SCEs from 24 to 72 hr.

Quantitative data on the meiotic study are presented in Table 2. Ara-C caused sperm-head

abnormalities after single and multiple dose administration with a peak at 1 week.



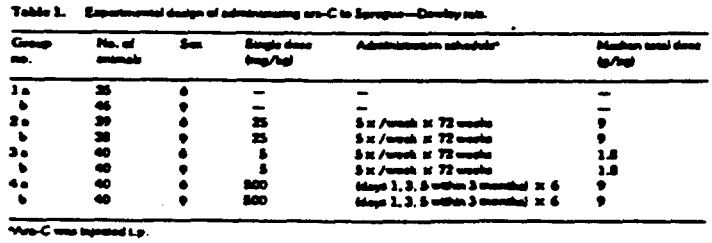
Ara-C exhibited a strong mitodepressive effect in human leukocytes in vitro as in Table 3. Ara-C induced dose-dependent structural abnormalities with a peak seen at 72 hr exposure. SCE analysis showed that a significant effect was noted for all doses from 24 to 72 hr exposure.

III. Carcinogenicity Studies:

A. Berger, MR and D. Schmahl Study on the Long-term toxic efficacy of cytosine arabinoside in Sprague-Dawley (SD) rats: Cancer Letters 43:59-64, 1988

Method:

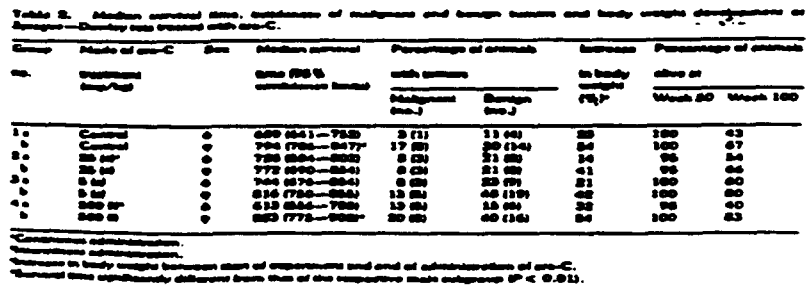
The test was conducted to determine the development of neoplasms following chronic administration of ara-C in SD rats. Animals were dosed as shown in Table 1.



Animals were observed twice daily, weighed monthly, and observed for life. They were sacrificed only when moribund. Dead animals were dissected and gross pathology and histopathologic examination were done for all detectable lesions.

Results:

Results indicated as in Table 2 below, in rats receiving identical doses (group 2 and 4), body weight gain was lower in the group that was treated daily. Ara-C treatment did not reduce the life-expectancy of the animals. Overall tumor incidence was slightly higher in male animals of groups 2, 3, and 4 and in female animals of group 4 than that of controls. The tumor incidence in the remaining animals was lower than controls.



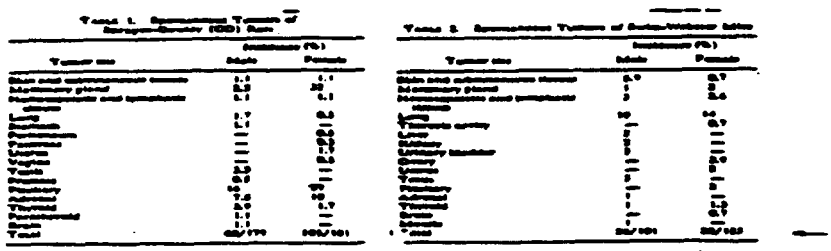
B. Weisburger, EK (1977) Bioassay Program for Carcinogenic Hazards of Cancer Chemotherapy. Cancer 40:1935-77

Method:

About 40 cancer chemotherapeutic drugs or combinations of drugs were selected for carcinogenic bioassay. A 90 day range-finding study with a 3 times per week dosing schedule for a total of 20 injections was conducted to estimate the MTD dose for the "carcinogenicity" study. For bioassay, 25/sex/group Sprague Dawley rats (CD strain) and Swiss mice (Swiss-Webster derived) received i.p. doses of each compound at the estimated MTD and ½ MTD three times weekly for 6 months. Animals were examined daily and weighed weekly for an additional 12 months. Animals that died before 100 days on study due to toxicity were not examined histopathologically. After 18 month, animals were killed and necropsied. Tissues and any apparent visible lesions were fixed for histopathologic examination. The tumors found in the experimental animals were tabulated for comparison with the control animals.

Results:

The control animals had an incidence of spontaneous tumors as shown in Table 1 & 2. For rats, the time to median tumor appearance was 1.5 yrs; 34% of the males and 58% of females had tumors, with a median survival time of over 18 months. The predominant tumors were mammary, pituitary and adrenal tumors in female rats whereas tumors of the endocrine organs were noted in males.



Forty-eight of 50 female rats survived 18 months after i.p. doses of 125 and 250 mg/kg Cytarabine (Ara-C). Eighteen of these 48 (38%) rats had tumors, including 10 breast (10/48=21%), 3 pituitary, and 2 adrenal. Six of the 18 tumors were malignant, but it was not specified in which tissue these originated.

In the control group, 58 out of 181 surviving females had mammary tumors (32%), the histology was not reported. If the level of significance is computed for 58/181 versus 10/48 treated rats with mammary tumors its p-value (0.156) is not statistically significant (Fishers exact test).

Compound	Animal	Dose	Mammary tumor incidence (%)
Control	Female rats	0	32
Cytarabine	" "	125-250 mg/kg	21

Forty-nine of 50 male rats treated with 125-250 mg/kg cytarabine survived 18 months. Thirteen of these 49 (27%) male rats had tumors, including 4 adrenal (4/49=8%), 2 pituitary, and 2 breast. Three of thirteen tumors were malignant, but it was not specified.

Only 10 of 50 female and 13 of 50 male mice survived 18 months after i.p. doses of 62 and 125 mg/kg Cytarabine (Ara-C). Two of the 10 surviving female mice had tumors (20%), including 1 lymphosarcoma and 1 uterine sarcoma. The tumor incidence in control female was 25% (38/153). Six of the 13 surviving male mice had tumors (46%), including 3 lymphosarcomas, and 2 lung. Tumor incidence in control male mice was 28% (28/101). However, tumor incidence is not an appropriate parameter for assessing carcinogenicity (McConnell).

Overall, there is no basis for concluding cytarabine is carcinogenic under the conditions of this experiment.

Summary and Evaluation:

The developmental toxicity of low doses of Ara-C was tested in mice. Pregnant Swiss mice were given i.p. doses of 0, 0.5, 2, or 8 mg/kg on days 6-15 of gestation. Administration of Ara-C during organogenesis produced maternal toxicity (i.e., lower weight gain and food consumption at 2 and 8 mkd) and developmental toxicity (i.e., cleft palate, deformed appendages, skeletal abnormalities). All fetuses in the 8 mkd group showed phocomelia and short and absent tail. Some fetuses in the 2 mkd group showed digital alterations. Fetotoxicity was observed by decreased fetal weight, decreases in the number of live fetuses and increases in early and late resorptions.

Teratogenic effects of Ara-C were determined after pregnant Swiss mice and SD rats were treated with s.c. doses of 12.5, 25, or 50 mg/kg/day on days of 16-18 or 18-21 of pregnancy, respectively. Animals exposed with 50 mkd Ara-C showed impaired cerebellum development (mice, rats), aggregates of poorly differentiated cells were indicative of defective nephrogenesis (mice), and marked focal microcystic renal changes and dilated tubules scattered in the renal cortex (rats). No abnormalities were found in the eyes of the treated mice, but in rats, marked retinal dysplasia was observed in all treated groups.

In prenatal treatment, a single i.p. dose of Ara-C at 50 mg/kg to pregnant rats on day 14 of gestation resulted in reduced litter weight and brain weight. In postnatal treatment, a single dose of Ara-C (50 mg/kg) at 5 days of age significantly reduced cerebellar weight and the ratio between cerebellar weight and brain weight. T-maze test was conducted on 15 week-old male rats whose mothers were treated prenatally with Ara-C (50 mg/kg). Ara-C offspring showed severe retardation of brain growth at 25 days of age. When compared to control rats, Ara-C rats made 80% more errors, indicating impaired maze learning ability.

Teratogenic effects of cytarabine were investigated in pregnant rats following a single i.p. injection of 2.5 to 900 mg/kg from day 5 to 12 of gestation. The lowest dose of cytarabine that produced malformations was 20 mg/kg on days 11 or 12. Malformations included cleft palate, micrognathia, and deformed rear appendages, paws and tail.

In mutagenic studies, the effects of Ara-C on the chromosomes of human leukocytes were tested. Ara-C (10^{-5}M) induced chromosome aberrations in human leukocytes and the effect consists of gaps and open breaks.

In a in vitro transformation study, exponentially growing HF (secondary Syrian Golden hamster fetal) and H43 (rat) cells were exposed to various doses of cytarabine for 2 to 24 hours. Transformed effects were induced by various doses (10^{-3} to 10^{-7}M) of Ara-C.

The effect of Ara-C was tested for the induction of micronuclei in mouse bone marrow erythrocytes. Ara-C was clastogenic at 12.5 mg/kg, i.p., and 25 mg/kg, p.o.

The cytogenetic effects of Ara-C were studied in in vivo and in vitro test systems. Male mice received either single or daily x 5 i.p. doses of Ara-C at 0, 160, 440, 660 and 870 ug. Animals were sacrificed at different time intervals after single and multiple doses. Ara-C exhibited a strong mitodepressive effect in mouse bone marrow cells. The effect was more intense in the multiple doses than single dose treatment. Cytarabine caused a dose-dependent increase in structural abnormalities, polyploidy, and SCEs with both schedules. Ara-C caused sperm-head abnormalities after single and multiple dose administration. In vitro, Ara-C induced dose-dependent structural anomalies in human leukocytes and in SCE analysis a significant effect was noted for all doses from 24 to 72 hr exposure.

Berger and Schmahl conducted a long-term carcinogenic effect of Ara-C in SD rats. Rats received either daily i.p. doses of 5 and 25 mg/kg or pulse doses of 500 mg/kg x 3/week for 72 weeks. Ara-C treatment did not reduce the life-expectancy of the animals. Overall tumor incidence (malignant/benign) was slightly higher in male rats treated with 25 mg/kg/day x 5 (38%), 5 mg/kg/day x 5 (35%) and 500 mg/kg x 3/week (87%) and female rats treated with 500 mg/kg x 3/week (50%) than control.

Weisburger tested about 40 cancer chemotherapeutic agents for their carcinogenic potentials. As part of the study, mice and rats were given i.p. doses of 125 and 250 mg/kg Ara-C three times a week for 6 months and observed for additional one year period. Forty-eight of 50 female rats survived 18 months. Eighteen of these 48 (38%) rats had tumors and 6/18 tumors were malignant (10 breast, 3 pituitary, 2 adrenal). If the level of significance is computed for control versus treated rats with mammary tumors its p-value (0.156) is not statistically significant (Fishers exact test). Thirteen of 49 male rats that survived had tumors and only 3/13 tumors were malignant. Only 10/50 female and 13/50 male mice survived 18 months after i.p. doses of 62 and 125 mg/kg Ara-C. Tumor incidences were 2/10 (20%) in female mice and 6/13 (46%) in male mice. However, tumor incidence is not appropriate parameter for assessing carcinogenicity. There is no basis for concluding ara-C is carcinogenic under the conditions of this experiment.

Recommendation: This NDA is approvable from the pharmacologic/toxicologic aspect of application with revision of the labeling as listed in this review.

Labeling Comments:

Labeling conforms to the format specified under CFR21. Part 201. Subpart B dated April 1, 1994. The proposed labeling describes the preclinical observations for the most part. However, the following revisions are requested:

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/S/
Doo Y. Lee Ham, Ph. D.

cc: Orig. NDA21041
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